iPhone Applications and Improvement in Weight and Health Parameters: A Randomized Controlled Trial.

by

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ABSTRACT

Dietary counseling from a registered dietitian has been shown in previous studies to aid in weight loss for those receiving counseling. With the increasing use of smartphone diet/weight loss applications (app), this study sought to investigate if an iPhone diet app providing feedback from a registered dietitian improved weight loss and bio-markers of health. Twenty-four healthy adults who owned iPhones (BMI > 24 kg/m^2) completed this trial. Participants were randomly assigned to one of three app groups: the MyDietitian app with daily feedback from a registered dietitian (n=7), the MyDietitian app without feedback (n=7), and the MyPlate feedback control app (n=10). Participants used their respective diet apps daily for 8-weeks while their weight loss, adherence to self-monitoring, blood bio-markers of health, and physical activity were monitored. All of the groups had a significant reduction in waist and hip circumference ($p<0.001$), a reduction in A1c ($p=0.002$), an increase in HDL cholesterol levels ($p=0.012$), and a reduction in calories consumed ($p=0.022$) over the duration of the trial. Adherence to diet monitoring via the apps did not differ between groups during the study. Body weight did not change during the study for any groups. However, when the participants were divided into low (<50% of days) or high adherence (>50% of days) groups, irrespective of study group, the high adherence group had a significant reduction in weight when compared to the low adherence group ($p=0.046$). These data suggest that diet apps may be useful tools for self-monitoring and even weight loss, but that the value appears to be the self-monitoring process and not the app specifically.

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CHAPTER 1

INTRODUCTION

Self-monitoring of dietary intake has been shown in multiple studies to be one of few successful strategies for weight loss and weight maintenance. Understanding the importance of self-monitoring stems from the social cognitive theory of self-regulation. Social cognitive theory states that human behavior is extensively motivated and regulated by the ongoing appraisal of self-influence, and that people cannot influence their own motivation and actions if they do not pay adequate attention to their own actions and the consequences that they produce (1). Social cognitive theory's key component is self-monitoring of one's behavior (2). Social cognitive theory can be utilized as an effective tool for weight loss, and incorporating self-monitoring into a weight loss plan is essential for success.

Multiple studies have reported on the efficacy of self-monitoring of dietary intake for weight loss and weight management. A study conducted on 123 postmenopausal overweight to obese women demonstrated that women who monitored their dietary intake on most days of the week experienced a 2.7% greater weight loss than the women who did not monitor their intake (3). A study conducted from 2007 to 2010 by Akers and colleagues tested self-monitoring on weight maintenance for 12 months following an initial weight reduction phase (4). After the initial weight loss period, study participants were randomized into two groups: a group that utilized self-monitoring techniques, and a control group (4). The results of the study determined that the group that utilized self-monitoring had an 87% greater weight loss than the control group after a 12 month period (4). In 2012, a study on the impact of self-monitoring of blood glucose on a behavioral

weight loss intervention for type 2 diabetic patients was published (5). The study found that the patients who were taught to use their diet to lose weight increased adherence to dietary recommendations when used in conjunction with the self-monitoring of blood glucose (5). These patients not only increased their weight loss but they also had better glucose control (5).

The traditional paper/pencil methods of self-monitoring is rapidly being replaced by technology, specifically hand-held devices such as personal digital assistants (PDA), smartphones, and iPads. A study conducted by Acharya and colleagues studied the use of a personal digital assistant (PDA) for self-monitoring versus the standard paper and pen method among overweight and obese adults (6). The study examined the difference in the two groups at six months (6). While both groups had significant reduction in weight, the PDA group increased consumption of fruit, vegetables, and decreased consumption of refined grains (6). These results lead researchers to believe that the use of a PDA for self-monitoring may improve self-awareness of dietary changes (6).

A study conducted at Arizona State University explored the use of smartphones and dietary tracking (7). The study assigned participants into three groups. A group utilizing an iPhone app to record and track dietary intake, a group using the memo function on the smartphone to input dietary tracking, and a group recording dietary intake via the traditional method of paper and pen (7). While all three groups had significant weight loss, the group using the iPhone app had greater adherence to self-monitoring when compared to the traditional paper and pen group (7). A follow-up focus group was conducted after these participants had completed the study to understand the participants' views regarding the use of the smartphones for dietary tracking (8). Overall, the

participants expressed the desire for feedback and social support in conjunction with the smartphone app (8).

A recent study investigated whether using a PDA with feedback was more effective than the PDA by itself, or the traditional paper and pen group for dietary self-monitoring and weight loss (9). Self-monitoring adherence was greater in both PDA groups when compared to the traditional paper and pen group (9). The greatest weight change was observed in the PDA with feedback group (9). Another study looked at the same parameters (paper & pen, PDA, PDA + feedback) as the above study but followed the study participants for 24 months (10). The results from this study showed that only the PDA + feedback group demonstrated significant weight loss at 24 months, suggesting that long-term weight loss requires feedback to maintain adherence to self-monitoring (10). A third study also explored the effect of technology on adherence to self-monitoring of diet and physical activity for weight loss. Of the three subject groups, paper & pen, PDA, and PDA with daily tailored feedback, the PDA and PDA + feedback intervention were associated with a significant weight loss through adherence to diet and physical activity self-monitoring (11). Additionally, the automated daily feedback messages available to the PDA + feedback intervention had an indirect effect on weight loss at 12 months (11).

While consistent self-monitoring of dietary intake improves weight loss outcomes in study participants; comprehensive nutrition and lifestyle education has been shown to improve dietary quality and weight loss in study participants as well. A randomized-controlled trial was conducted on obese Hispanic Americans following Roux-en-Y gastric bypass surgery. Participants were randomized into a comprehensive nutrition and

lifestyle intervention group (n=72) or a non-comprehensive approach group (n=72) (12). Twelve months after surgery, both groups had significant weight loss, but the comprehensive intervention group experienced greater weight loss and a larger reduction in BMI (12). The comprehensive intervention group also had a higher mean intake of protein and participated in physical activity more often (12).

In another study, a female NCAA volleyball team was recruited to determine if nutrition education provided by a registered dietitian improved the dietary intake of the team (13). At the conclusion of the study, a significant improvement in dietary intake and nutrition knowledge was evident for the volleyball team following the education intervention by a registered dietitian (13).

At this time, there is no available published research on the use of a smartphone app with feedback from a registered dietitian for dietary intake tracking and weight loss. Mobile phones offer a multitude of functions and are with the user throughout the day. The purpose of this study was to compare the use of a new dietary self-monitoring smartphone app (MyDietitian) with and without the feedback of a registered dietitian. We also compared both of these groups to a feedback control group that utilized a dietary self-monitoring smartphone app that encouraged adherence to the USDA's MyPlate dietary guidelines. We hypothesized that the use of the smartphone app with feedback from a registered dietitian would provide significant improvement in weight loss and bio-markers of health when compared to the non-feedback and feedback control groups. We also hypothesized that the use of the smartphone app with feedback from a registered dietitian would improve adherence to dietary tracking when compared to the non-feedback and feedback control groups. Finally, we hypothesized that the use of the

smartphone app with feedback from a registered dietitian would improve diet quality when compared to the non-feedback and feedback control groups.

Delimitation

Only motivated individuals that were willing to record their dietary intake with a smartphone, specifically an iPhone participated in this trial. This study did not address populations that either do not own or are not willing to utilize a smartphone. This study also did not address the population of individuals that own a smartphone brand other than an Apple iPhone. This study included only adults that live in the Southwest. This study cannot be generalized to children, elderly individuals, and people that live in other parts of the United States or the world.

Limitations

Adherence to protocol may not be consistent among participants over the 8-week trial. Also, since multiple registered dietitians provided feedback to the participants, there may not have been complete consistency in terms of counseling and nutritional information provided to each of the participants.

Definitions

Overweight- defined as a body mass index of 25.0-29.9 kg/m^2

Obese- defined as body mass index of 30.0 kg/ m^2 and above.

PDA- defined as a personal digital assistant of hand-held computer device.

CHAPTER 2

REVIEW OF LITERATURE

Obesity: The Problem

The prevalence of overweight (BMI 25-29.9) and obese (BMI greater than or equal to 30.0) individuals in the United States rose drastically from the late 70's through the early 2000's, but in recent years the increase has leveled off (14). Results from the 1999-2000 National Health and Nutrition Examination Survey (NHANES) estimates that 64% of adults in the U.S. are either overweight or obese (15). Specifically, 35.7% of adults are obese (14). Obesity is related to multiple health conditions including heart disease, stroke, certain types of cancers, and type 2 diabetes (14). The Centers for Disease Control and Prevention (CDC) estimate that in 2008 the medical costs associated with obesity were $147 billion (14). The CDC also determined that the medical costs paid by a third-party payer for people that were obese was $1,429 higher than that of a person of normal weight (14).

A study conducted in 2003 examined the attitudes of primary care physicians about obesity and its treatment. The study sent a questionnaire by mail to 5000 primary care physicians (16). Half of the questionnaires (n=2500) classified obesity as a BMI of greater than 40 kg/m2; while the other half (n=2500) of the questionnaires classified obesity as a BMI of 30 to 40 kg/m2 (16). The different classifications of obesity were used to assess any effect of the degree of obesity on physicians' attitudes (16).Six hundred and twenty out of the five thousand primary care physicians responded to the survey (16). The survey asked the physicians questions on several areas of obesity: beliefs about treatment, causes of obesity, attributes of obese individuals, weight loss

outcomes, and efficacy of obesity treatments available. The physicians that responded to the survey rated physical inactivity as the most important cause of obesity. The next highest rating for the cause of obesity was overeating and a high-fat diet (16). Only 49% of the physicians felt competent in prescribing weight loss to their patients and 54% stated that they would spend more time working on weight related issues if they were able to be financially reimbursed for their time (16). Of the physicians polled at least 50% viewed their obese patients as unattractive, ugly, noncompliant, and awkward; while about one-third consider the obese to be weak-willed, lazy and sloppy (16). Only 14% of the physicians polled felt that they were able to help the obese lose weight (16).

The physicians, when asked if the efficacy of obesity treatment was as effective as the treatment of 10 other chronic conditions (hypertension, asthma, coronary artery disease, hyperlipidemia, diabetes, depression, osteoarthritis, cigarette smoking, alcoholism, and drug addiction), felt that the treatment of chronic conditions except drug addiction was more effective than the treatment of obesity (16).

The physicians in the previous study believe that physical inactivity is the most important cause of obesity. If physical inactivity is the main cause of obesity then using increased physical activity as a means to treat obesity should be rather effective. A study conducted by Dahlkoetter and associates randomly assigned forty-four subjects to one of four groups: exercise, eating habits, combination, and control (17). The study had a total of forty-four women, between the ages of 16 and 50 years of age, at least 15 pounds overweight, and had medical clearance if there were any underlying medical conditions (17). The exercise intervention group focused primarily on ways they could improve physical fitness and expend more energy through exercise (17). The exercise group like

the other two groups was educational in nature and did not consist of constant supervised exercise (17). The study consisted of an initial meeting, an 8-week intervention, and 8-week follow up and then an extended 6 month follow up (17). The combination of exercise and eating habits group had the greatest improvement in body circumference and weight loss (17). The combination group was also the only group that continued to lose week at the eight-week follow-up appointment (17). The results from this study lead researchers to believe that exercise alone will not allow individuals to meet their weight loss goals.

In 2006 a review was conducted by Drs. Jakicic and Otto to look specifically at the role of exercise in the treatment and prevention of obesity (18). This review noted that well known studies have shown a relationship between the amount of leisure-time physical activity and risk for all-cause mortality (18). While it is clear that physical activity can help mitigate the increased all-cause mortality risk of obesity, it is not clear that exercise alone can treat obesity (18). Weight loss that is achieved through exercise alone is far less than what can be achieved by significant reductions in energy intake (18).

A study conducted by Caudwell et al. in 2009 explored the relationship between diet, exercise, and weight loss. The study participants were fifty-eight obese men and women with a mean BMI of 31.8±4.5 kg/m2 (19). These participants were assigned an exercise regimen that expended approximately 500kcal per session five times per week for twelve weeks (19). The participants had anthropometric characteristics and total daily energy intake assessed at weeks 0, 4, 8, and 12 (19). The researchers found that while there was a significant reduction in weight over the trial ($p<0.001$), there was a large individual variability in weight loss between the participants (19). The researchers further explored

the dietary habits of the individuals that lost an insignificant amount of weight or gained weight and found that those individuals dietary intake changed from baseline to completion of the trial (19). These individuals increased their daily energy intake over the twelve weeks ($p=0.043$) while reducing their fruit and vegetable consumption ($p=0.005$) and increasing fat consumption (19). The individuals that lost significant amounts of weight did not increase their fat consumption, decreased energy intake, and increased their fruit and vegetable consumption ($p=0.005$) (19). This finding by Caudwell et al. suggests that in order for an exercise program to successfully reduce weight a reduced calorie diet is also required to have significant weight loss.

A meta-analysis conducted in 1997 looked at the previous twenty-five years of research on weight loss by diet, exercise, or diet plus exercise. The meta-analysis included 493 studies. The researchers found that exercise alone did not produce significant weight loss, and reduction in body fat (20). The diet and diet plus exercise groups produced significantly better reductions in weight and body fat when compared to the exercise alone groups ($p<0.05$). The diet plus exercise group had the highest level of weight and body fat reduction although the results were not significantly different from the diet alone group (20). The results from this meta-analysis suggest that when the goal is weight loss and body fat reduction diet or diet plus exercise is more effective than exercise alone (20).

Established Treatments:

Behavior Modification

Behavior interventions are often used in the treatment of obesity. Foster et al. reviewed the key components of the behavioral treatment of obesity in the *American*

Journal of Clinical Nutrition. Behavioral treatment of obesity helps individuals obtain a set of skills that can be used to achieve weight loss and weight maintenance (21). Behavior therapy has the ability to help an individual identify triggers in their lives that bring on unhealthy eating habits, and gives them a set of skills to help the individual respond differently when these triggers occur (21). Behavior treatments also incorporate cognitive therapy techniques which teach individuals how to set realistic goals for weight loss, to have more realistic expectations of progress, and coping techniques when their goals are not met (21). The cognitive therapy techniques used to treat obesity are based on the therapies developed for the treatment of eating disorders, anxiety, and depression (21).

The treatment of obesity with a behavioral focus has several key components. The treatment is goal directed and the goal needs to be easily measurable by the individual and the counselor so that achievement of the goal can be clearly monitored (21). The individual is also expected to develop behavior change skills which can be used regularly so that as the individual learns to utilize the behavior change skills and success becomes a matter of skill use and not sheer will power to stay on a diet or exercise program (21). Behavior change models have been widely validated but there is a need to identify which aspects of a standard behavior change intervention have the greatest effects on obesity (22, 23). The interventions usually include food diaries, nutrition education, physical activity, problem-solving, stimulus control, portion control, mindful eating, and cognitive therapy (21).

The role of a behavioral counselor was reviewed by Foreyt et al. in 1998. The reviewers state that the first goal of a behavioral counselor for weight reduction should be

to establish a collaborative relationship with the individual being treated (24). The research has shown that when an individual has a strong relationship with their counselor there is an improved treatment outcome and less resistance to change from the patient (24). The reviewers state that if the counselor does come up against resistance from the patient, the counselor should use that resistance to make an assessment of the patient's readiness to change, and possibly leading the counselor in a direction that is better suited for that particular individual (24). The reviewers focus on the need to individualize treatment and that success can be measured by other means than just weight loss such as: improvements in physical activity, metabolic profiles, self-esteem, body image, functional capacity and quality of life (24). The review of the role of behavior counselor states that registered dietitians can effectively fill this role due to their extensive training in nutrition knowledge and training in nutritional behavior change counseling (24).

A randomized control trial examined how to strengthen the effectiveness of behavioral interventions for weight loss in 202 research participants half being women (n=101) and half being men (n=101) (25). Subjects were randomized into one of five groups: control, standard behavioral treatment (SBT), standard behavioral treatment plus food provision (SBT + FP), standard behavioral treatment plus incentives (SBT + I), and standard behavioral treatment plus food provision and incentives (SBT + FP + I) (21). The standard behavioral treatment included weekly counseling in small groups (about 20 individuals at a time) for the first twenty weeks and then once per month until the 18 month follow-up (25). The sessions were led by trained interventionists with advanced degrees in behavioral science or nutrition (25). The program emphasized several behavior modification techniques such as: problem-solving strategies, social assertion, stimulus

control techniques, short-term goal-setting and reinforcement techniques for enhancing motivation, cognitive strategies for replacing negative thinking, social support, and relapse prevention (25). The standard behavioral treatment was identical for all four groups that included SBT as part of the intervention. There were two groups that included food provision as a treatment intervention. The food provision provided consisted of prepackaged meals for five dinners and five breakfasts each week for the 18-month duration of the trial (25). These participants were also provided with a meal plan with recipes (25). The intervention groups that included an incentive were given a cash payment based on the amount of weight lost that week in relationship to their overall weight loss goal (25). The minimum payment per week was $2.50 and the maximum payment was $25 per week (25). The results of the study showed that the the SBT plus food provision group lost 1/3 more weight than the standard behavior treatment group at the six month mark (25). The SBT plus food provision also had 100% greater weight loss than the standard behavior treatment group at the 12 month mark and 40% greater weight loss at 18 months (25). The SBT plus food provisions and incentives had the second greatest weight loss of an average loss of just over 6kgs at 18 months (25). The study found that there was no significant effect on weight change for providing financial incentives, and that the significant reduction in BMI was due to providing food provisions to the participants (25). When analyzing the findings of this study several key components stood out as possible explanations as to why the SBT plus food provision group fared better than the other groups. The food provision participants completed more components of the assigned standard behavior treatment assigned. These participants completed more food and exercise diaries, attended more SBT meetings, had greater ease

in estimating caloric content of the food eaten, and overall had greater adherence to the program than that of the other groups (25). Greater adherence to the program and greater adherence to food and exercise diaries may be why the SBT plus food provision group had a greater weight loss than the other three groups.

The National Weight Control Registry (NWCR) was established in 1994 to investigate the behaviors of individuals that succeed at long-term weight loss. (26) The NWCR has identified key behavioral strategies of individuals that have successfully maintained weight loss. Consistent self-monitoring of weight, self-monitoring and recording of dietary intake, including exercise daily, and consuming a diet low in fat and calories were associated with successful weight loss and long-term weight maintenance. (27)

Self-Monitoring

A recent publication aimed to examine more closely diet strategies for weight loss. The authors conducted a secondary study to the Nutrition and Exercise for Women study (3). The Nutrition and Exercise for Women study was a four-arm randomized control trial that combined effects of dietary weight loss and exercise-based weight change on overweight to obese postmenopausal women (3). The researchers examined the strategies used for successful weight loss in the Nutrition and Exercise for Women study and only included the women who completed the 12-month trail (3). This included a total of 123 women with 59 of them being randomized to the diet group and 64 being randomized into the diet and exercise group (3). The secondary study found that nine specific behaviors were significantly associated with the percent of weight change at the twelve month mark (3). They were: change in percent energy from carbohydrates, change

in percent energy from fat, using food journals continuously, measuring foods, monitoring energy intake, eating out for breakfast, eating out for lunch, eating out for dinner, and skipping meals (3). Most notable were the results from women who complied to journaling their food intake. The women who were in the 75th percentile for food journaling had a 3.7% greater weight loss than the women in the 25th percentile for food journaling (3). These women also had a 2.7% greater weight loss than the women who did not comply with food journaling (3).

In 2010, Akers et al. tested self-monitoring of body weight, fruit/vegetable intake, water consumption and step count for weight maintenance after weight loss. The participants in this study completed an initial 12-month weight loss intervention where they were randomly assigned to an intervention group which consisted of a 1200 to 1500 kcal diet plus 16 fl oz of water prior to each meal (WEV+) or a control group which consisted of 1200 to 1500 kcal diet alone (WEV) (4). The initial 12-month weight loss intervention did not include self-monitoring or self-regulation strategies (4). During the subsequent weight maintenance intervention the groups maintained their status of either having additional water before meals or no additional water requirements (4). Both groups were directed to track body weight, fruit/vegetable intake, and step count on tracking sheets during the weight maintenance intervention with the WEV+ group additionally tracking water consumption (4). The study found that while there was not a difference between the groups, the participants that had the greatest adherence to dietary tracking maintained the greatest weight loss (4). The overall compliance for returning the tracking sheet was 76% ± 5%, leading researchers to believe that dietary tracking is a feasible approach to weight loss maintenance (4).

Self-monitoring of dietary tracking has been traditionally done with a paper and pencil method. This traditional method is being rapidly replaced by electronic hand-held devices like personal digital assistants (PDA), smartphones, and iPads. A recent study conducted by Acharya et al. studied the use of a personal digital assistant (PDA) for dietary self-monitoring among overweight and obese adults versus the standard paper and pencil method in the same population (6). The participants (n=210) were randomized into three groups at the start of the study (6). The groups were a standard paper record (PR), a PDA with dietary and exercise software with customized feedback (PDA+FB), and a PDA with just the dietary and exercise software (PDA) (6). At the end of the trial no difference was found between the PDA and PDA+FB groups so they were combined to compare against the standard paper record group (6). All study participants were provided with a cognitive-behavioral intervention in the form of 20 group sessions during the first six months of the trial (6). Every participant was trained to use their specific self-monitoring tool during the initial two weeks of the intervention (6). During each of the twenty group sessions the standard paper record group turned in their records and the researchers downloaded the data from the PDA groups (6). The study monitored tracking adherence weekly by evaluating if the participant consumed (via diet records) more than 50% of the weekly calorie goal, and if the participant accomplished this they were considered adherent to dietary tracking for that week (6).

At the six month completion mark of the trial 91% or 192 participants of the original 210 completed the 6-month assessment (6). The breakdown of the groups at the six month mark was as follows PR=63, PDA (both groups combined) =129 (6). While both groups had significant weight loss at the completion of the trial, the PDA group

increased their servings of fruits and vegetables and decreased consumption of refined grains significantly (6). The standard paper record group also had a significant decreased total fat intake with increased adherence to self-monitoring (6). The researchers from this study believe that greater adherence to self-monitoring might assist people with becoming more aware of their consumption choices (6).

While the study above combined the PDA and PDA+feedback group at the conclusion of the study due to statistical insignificance between the groups another study sought to find out if a standard paper record, a PDA, or a PDA with feedback was more effective for self-monitoring adherence. This study conducted by Burke et al. used the 6-month assessment data from the SMART Trial which was a 24-month single-center randomized control trial for the behavioral treatment of weight loss (9). All of the intervention groups received standardized behavioral intervention which included: weekly exercise goals, daily dietary goals, groups sessions, and daily self-monitoring of eating and exercise (9). The standard paper record group (PR) was given standardized paper food diaries and given instructions to record the calories and fat grams of foods, all food consumed, and minutes of exercise completed (9). The PDA groups were both provided with Palm Tungsten E2 PDA's that included dietary tracking software, and the PDA plus feedback group had customized software that provided daily feedback messages based on an algorithm from the participants dietary entries (9). This feedback focused on positive reinforcement and guidance on how to obtain goals (9). The participants were monitored for self-monitoring adherence weekly (9). If the participant met more than 50% of the weekly caloric goals the participant was counted as adherent for that week (9). In addition, if the standard paper record group simply did not turn in

the paper record, they were classified as non-adherent for that week (9). The results of this study showed that all three groups had a statistically significant weight loss ($P<0.01$), and there was not a significant difference between the groups (9). While there was not a significant difference between the groups, the PDA+FB group (63%) had a higher percentage of weight loss greater than or equal to 5% (9). The paper record group had 46% achieve greater than or equal to 5% weight loss, and the PDA without feedback had 49% achieve greater than or equal to 5% weight loss (9). The adherence to self-monitoring was also significantly greater in both of the PDA groups than in the standard paper record group (9). All of the groups lost a significant amount of weight, but only the PDA+FB group had the largest percentage of participants lose greater than 5% body weight (9).

The same group of researchers conducting the SMART Trial described above continued to monitor the participants for a 24-month period (10). This publication compared only the PDA group and the PDA+FB group at the 24-month conclusion of the trial (10). The intervention groups received standardized behavioral intervention which included: weekly exercise goals, daily dietary goals, groups sessions, and daily self-monitoring of eating and exercise (10). Both groups were both provided with Palm Tungsten E2 PDA's that included dietary tracking software, and the PDA plus feedback group had customized software that provided daily feedback messages based on an algorithm from the participants dietary entries (10). Only the PDA + feedback group demonstrated a statistically significant weight loss at the 24 month mark, suggesting that long-term weight loss requires feedback to maintain adherence to self-monitoring for weight loss (10).

In 2012, The Cochran Collaboration published a review article on interactive computer-based interventions for weight loss or weight maintenance in overweight or obese people. (28) The review included four weight maintenance studies (n=1603) and 14 weight loss studies (n=2537). The review only included articles containing randomized or quasi-randomized controlled trials that studied interactive computer-based weight maintenance or weight loss. The participants were overweight or obese adults, and the duration of the trial needed to be at least four weeks (28). The studies included had a treatment duration between four and 30 months (28). The studies reviewed had a computer-based intervention group, a minimal intervention group (control, usual care, pamphlets), and an in-person treatment group (28). The computer-based interventions had a greater weight loss than a minimal intervention (usual care, pamphlets) at six months with a mean difference of -1.5kg (95% CI -2.1 to -0.9) (28). The computer-based interventions also had a greater effect at limiting weight regain when compared to the minimal interventions at the six month mark with a mean difference of -0.7kg (95% CI -1.2 to -0.2) (28). While the computer-based interventions were superior to a minimal intervention, the in-person treatment group was superior to both the minimal intervention and computer-based interventions suggestion that direct feedback from a professional may be more effective for losing and maintaining weight loss in overweight or obese adults (28).

Nutrition Education

Comprehensive nutrition and lifestyle education has been found to improve weight loss and physical activity (12). A randomized controlled trial from 2008-2010 that included 144 Hispanic Americans following Roux-en-Y gastric bypass surgery looked at

the effectiveness of a comprehensive nutrition and lifestyle intervention conducted over a 12 month time period (12). The study participants were randomized into one of two groups: the comprehensive nutrition and lifestyle intervention (n=72) or the non-comprehensive approach standard care (n=72) (12).

The comprehensive intervention group received six nutrition and lifestyle education classes (12). The classes were in groups of up to 12 individuals and were provided in either English or Spanish following the individual's language preferences (12). Each session was approximately 90 minutes in duration. In the first session the intervention group received a meal planning guide and a diet, including characteristics of a Hispanic diet and modifications specific to the Hispanic culture (12). The session also included tips for controlling portion sizes, recommendations for avoiding unhealthy foods, and an exchange list for weight management (12). The diet provided to the intervention participants limited calories to 1,400 kcals and recommended a minimum daily protein intake of 60-70g (12). The second session focused on the importance of physical activity after weight loss surgery (12). Session 3, 4, 5, and 6 focused on emotional support and behavior change strategies for weight loss and weight maintenance (12). The comprehensive intervention group also received reminders by email and telephone encouraging them to adhere to self-monitoring of dietary intake and physical activity (12).

The results of this program showed that twelve months after surgery both groups had significant weight loss, but the comprehensive nutrition and lifestyle intervention group experienced a larger reduction in BMI (CN&L 6.48+/-4.37 vs. standard care 3.63 ± 3.41; $P<0.001$) and had an overall greater excess weight loss (CN&L 80% preoperative

excess weight vs. standard care 64%, P<0.001)(12). The researchers also found that the comprehensive nutrition and lifestyle intervention group had a higher mean intake of protein (P=0.02) and participated in physical activity more often than the non-comprehensive group (CN&L +14min/wk vs. standard care -4 min/wk; P<0.001) (12). These results lead researchers to believe that a comprehensive nutrition and lifestyle education program could be effective in assisting obese individuals to lose weight while having a better diet quality (12).

A feasibility study conducted on overweight and obese breast cancer survivors explored the use of a lifestyle intervention to reduce the recurrence of cancer and the development of other chronic diseases (29). The study participants (n=14) were women >18 yrs of age, a BMI 25-35, diagnosed with Stage I to IIIa breast cancer in the previous 5 years, completed chemotherapy/radiation therapy for at least 3 months, and could fill out study questionnaires in English (29). The exclusion criteria were as follows: plans to join an organized weight loss program, if the participants was already participating in >150 min/wk of moderate to vigorous activity in the past six months, has uncontrolled diabetes, or had any indications that treadmill testing or entry into a diet/exercise program could not be completed by the participant due to health complications (29). The study utilized a single group design to assess the efficacy of the intervention (29). The diet intervention was conducted by a registered dietitian (29). The intervention consisted of 16 group-based sessions and the curriculum followed the Diabetes Prevention Program model (29). The 16 sessions were held over a 24-month period with the sessions being once per week for the first eight weeks and every other week for the duration of the trial (29). The sessions were led by registered dietitians with at least 15 years of experience

working with breast cancer survivors (29). The diet intervention sessions provided specific strategies to reduce energy intake such as food diaries and daily weighing (29). The results of the study showed that the participants lost a mean of 3.8 ± 5.0kg ($p= 0.01$), reduced BMI by 1.4 ± 1.9 ($p=0.01$), reduced total body fat percent by 2.4 ± 2.7% ($p<0.01$), reduced waist circumference by 4.2 ± 6.6cm ($p=0.03$), and reduced hip circumference by 5.5 ± 5.3 ($p<0.01$) (29). The results of this feasibility intervention has lead researchers to believe that a diet and lifestyle intervention led by registered dietitians could be a feasible strategy to improve outcomes in overweight and obese cancer survivors (29).

The Diabetes Prevention Program was a 27-center randomized controlled trial conducted in the United States (30). The trial evaluated the efficacy and safety of interventions in preventing or delaying diabetes in high-risk individuals (30). The trial compared three treatment groups that were: standard care plus metformin, standard care plus placebo, and intensive lifestyle modification (30). Dietitians were considered an integral role in the programs application (30). The lifestyle intervention consisted of a 16-module lifestyle change curriculum, and each participant was to complete this curriculum with a dietitian on an individual basis in the first 24 weeks of participation in the study (30). The curriculum included sessions on self-monitoring of diet and exercise, reducing fat intake, strategies for eating away from home, stress management, healthy eating to prevent diabetes, and ways to increase physical activity (30). Once the participants completed the first 24 weeks they moved onto a post-core curriculum phase (30). In this phase the dietitian was to contact the participant at least once per month to discuss weight loss intervention goals (30).

The dietitians were able to tailor the intervention to the individual at this stage. While the dietitians were required to meet with the participant at least once per month, the dietitian had the freedom to meet with the participant once per week if that is what they felt was necessary for the participant's success in the program (30). Some participants received weekly sessions, while others only met once per month. Each dietitian used their individual counseling skills to build a bond with the participants to encourage the participants' success in overcoming their individual barrier to change (30). The dietitians tailored post-core classes to meet the needs of each individual participant, and the dietitian could choose from an approved list of classes provided to each center or create their own post-core class (30).

The results of the diabetes prevention program showed that the intensive lifestyle intervention group achieved a mean weight loss of 7% at 1 year of intervention and maintained a 5% weight loss at 3 years (30). The lifestyle intervention group had an average weight loss of 5.6 kg while the metformin group had an average weight loss of 2.1 kg, and the placebo group had a loss of 0.1 kg (31). These results lead researchers to believe that dietitians can play a pivotal role in helping overweight and obese individuals meet their health and weight loss goals through a prescribed curriculum of diet and lifestyle strategies that can be individually tailored for different participant needs.

Another study looked at the efficacy of a dietitian intervention and lipid values in hyperlipidemic men with a history of niacin non-compliance. Niacin is widely prescribed for patients with a combined hyperlipidemia since it lowers VLDL and LDL while increasing HDL (32). Niacin is also poorly tolerated in some patients and can have a high non-compliance rate due to adverse side effects (32). The studies main outcome measures

were serum total cholesterol, low-density lipoprotein cholesterol, triglycerides, if the cost of medical nutrition therapy for 1 year would be less than lipid-lowering medications, and to evaluate if eight weeks of medical nutrition therapy is effective enough to remove a patient from lipid-lowering medications (32). The study looked at medical records of 73 male veterans with hyperlipidemia and then screened by telephone for niacin compliance (32). The number of subjects that completed the study was 43 (32). The subjects that self-reported discontinued use of a prescribed niacin regimen were then determined to be non-compliant (32). The participants had dietitian intervention visits at week 0, 2, 4, 6, and 7 (32).

The study resulted in significant reductions in total cholesterol, low-density lipoprotein cholesterol, total triglycerides, and BMI from baseline (32). There was not a control group in this study (32). There was also a significant increase in high-density lipoprotein levels (32). The researchers also conducted a cost savings analysis and found that medical nutrition therapy provided $638.35 net cost benefit per patient when compared with statin therapy (32). This study illustrates the efficacy of dietitian intervention when compared with pharmacology or standard care in hyperlipidemic patients.

A 12-month study conducted in 2004, by Wolf and associates evaluated a dietitian-led intervention to reduce waist circumference and weight in obese patients with type 2 diabetes. The study provided either a registered dietitian intervention (n=74) or standard care (n=73) (33). The participants that were assigned to a registered dietitian intervention received a total of 6 individual meeting, 6 one-hour small group sessions, and telephone communication with the dietitian case manager to assess waist

circumference, weight, lab results, patient care issues with physician, goal-setting, and nutrition education (33). The standard care participants were provided with educational materials and given the freedom to belong to other weight loss or diabetes care programs (33).

The results of the study indicated that the dietitian-led intervention group improved significantly over the standard care group in several areas (33). The dietitian intervention group had a mean weight change of -2.4kg, while the standard care group had a mean change of +0.6kg (33). The dietitian intervention group also had a mean change of -5.5cm for waist circumference while the standard care group had a mean change of -1.4cm (33). The dietitian intervention group also took fewer prescription medications per day ($p=0.03$). The health related quality of life questionnaire also indicated that the dietitian intervention group improved in 7 of the 9 domains when compared to the standard care group ($p<0.05$) (33). These results indicate that a registered dietitian-led intervention is superior to standard care in obese patients with type 2 diabetes (33).

Another study involving diabetic participants looked at the effect of the use of dietitian education on metabolic and cardiovascular health after a 24 month intervention (34). The participants studied were assigned to a control group with conventional endocrinologist follow up (n=50) or an on-site dietitian education intervention provided every 3 months with an annual endocrinologist follow up appointment (n=51) (34).

The results of the study showed that weight ($p=0.04$), BMI ($p=0.009$) and waist circumference ($p=0.01$) were significantly different between the control group and the dietitian education group (34). Hemoglobin A1C was reduced significantly in the

dietitian education group when compared to the control group (p=0.04) with a drop of -0.6% vs. the control -0.3% (34). The dietitian education group had improved energy intake as well with -548 kcal/day vs. the control -74 kcal/day (p=0.04) (34). The results from this study indicate that dietitian education should serve, at least in part, as standard care with annual endocrinologist follow-up to reduce risk of cardiovascular disease in diabetic patients (34).

A study conducted by Welty et al. examined the effect of onsite dietitian counseling of weight loss and lipid levels in an outpatient physician office (35). The study utilized a weight-loss program that focused on assessment of cardiovascular risk factors and lifestyle changes that included diet and exercise (35). The program used dietitian counseling on two occasions (35). The study stressed that the dietitian visits in this study are fully reimbursable and have strong implications for a cost-effective strategy to combat obesity (35).

The study included eighty overweight or obese patients, and all of the participants were assigned to treatment (35). This study did not utilize a control group, and the results of this study are significant (35). The participants (n=64) lost a total of 5.6% of total body weight at a mean follow-up point of 1.75 years, and 81% of participants maintained an average weight loss of 5.3% at 2.6 years (35). The participants also improved their lipid profile by lowering LDL cholesterol by 9.3%, increasing HDL by an average of 9.6%, and lowering triglycerides by an average of 34% (35).

These data show that dietitian education provided in an outpatient physician office provides significant reductions in weight and improvements in lipid profile (35).

The intervention utilized is also a fully reimbursable service indicating that cost-effective reimbursable approaches are available to combat obesity (35).

Linking Treatments with Technology:

Social Cognitive Theory and Technology

Social cognitive theory is frequently used in weight-loss and physical activity interventions (36). Social cognitive theory asserts that the less individuals are aware of how their lifestyle affects their health, the less likely the individuals are to change those lifestyle factors (36). Knowledge of the effects of one's behavior creates a precondition for change, but self-influences are necessary to overcome the barriers to adopting new lifestyle behaviors (36).

The strongest self-influence, according to social cognitive theory, is perceived self-efficacy (36). Self-efficacy is one's belief that they are capable of completing a task (36). Having self-efficacy that one can complete a task or exercise self-control can make the difference between losing self-control and exercising it (36). Self-efficacy regulates motivation by influencing the goals individuals set for themselves (36). It also determines the commitment to the goal and the expectations the individual has when they reach that goal (36). The individual's personal belief that they have the power to achieve their goals determines how long an individual will continue to try to reach that goal in the face of obstacles and failure (36).

Bandura has determined that there are four main sources of influence when developing beliefs about self-efficacy. The first source, and strongest, is through mastery experiences (36). Mastery experiences build a portfolio of successes and this strengthens a person's level of self-efficacy (36). The successes must come from experiences that

challenge the individual by allowing them to overcome obstacles by persistent effort (36). If the success comes quickly and without obstacles the individual will expect quick results and will be easily overcome by failures (36).

The second source of building self-efficacy is through vicarious experiences (36). This source is also known as modeling. This is where an individual in a social situation sees a similar individual have success achieved by sustained effort to reach a goal (36). Modeling provides a social standard where an individual can judge their own capabilities as well as the model can teach the observer effective strategies for reaching a goal with environmental obstacles (36).

The third source of building self-efficacy is through social persuasion (36). If an individual is persuaded verbally that they are capable of mastering a given task they are more likely to put forth greater effort to accomplish that task (36). The fourth source is an individual's reliance on their somatic and emotional states when making decisions on their self-efficacy to complete a task. A person will evaluate their stress response, mood state, and strength and stamina when assessing their ability to complete a task (36). In order to increase an individuals' self-efficacy it is important to foster positive mood states, and reduce physical and mental stressors (36). Social cognitive theory requires that individual's set accurate goals and monitor their behavior in order to achieve those goals (36).

A study conducted by Palmeira et al. explored predicting short-term weight loss and the four leading health behavior change theories. The study subjects were overweight or obese women (n=142), older than 24 years of age, premenopausal and not currently pregnant, free from major disease, and have a BMI greater than 24.9 kg/m2 (22). The

women were randomized into one of four groups: Social Cognitive Theory (SCT), Theory of Planned Behavior (TPB), Transtheoretical Model (TTM), and Self-Determination Theory (SDT) (22). The researchers found that the SCT and TTM groups represented the strongest models for weight management ($p<0.001$). In the SCT group changes in self-efficacy accounted for 20.5% of the weight change variance (22). Both the SCT and TTM theories include self-efficacy within the behavior change model (22).

A web-based randomized controlled trial was conducted by Collins et al. This study evaluated a commercial web-based weight loss and weight maintenance program in overweight and obese adults (23). The web-based weight loss and weight maintenance program was designed around the social cognitive theory of behavior change (23). The key components of social cognitive theory that were targeted in the program were: self-efficacy (goal-setting, self-monitoring, body measurements, exercise, and diet), outcome expectations (knowledge of web-based components), modeling (interactive website demonstrations and features), and social support (forums, blogs, email contact, and feedback) (23). The participants were randomized to a control, basic, and enhanced program groups (23). The control group was a 12-week wait list; at the end of 12 weeks these participants were then randomized into one of the intervention groups. The basic group received free access to the study website with the following features: individualized daily calorie targets, online food and exercise diaries, weekly menu plans with a grocery list, weekly educational tips and challenges, online forums for social support, daily and weekly calculations of nutrition summaries and energy balance, weekly newsletters, self-monitoring of body weight and waist circumference reminders, goal-setting options with graphical display (23). The enhanced group received the basic

features plus these additional features: a personalized enrollment report which suggests weight loss goals and key behaviors the participant will need to change to reach the goals, weekly automated personal feedback for nutrition and exercise levels based on the participants entries into the online diary, weekly feedback on their current level of weight loss success, and a reminder schedule for diary compliance (23). The results showed that the basic and enhanced groups lost a significant amount of weight and had a significant reduction in waist circumference when compared with the control groups (23). The study also found significant between-group differences in the percentage of participants who lost greater than or equal to 10% of their baseline weight (control: 0%, basic: 18%, enhanced 28%; $p<0.001$), suggesting that the enhanced version of the web-site allowed participants to lose the greatest amount of weight (23). The enhanced version group also had the least amount of participants that gained weight at 17% ($p<0.001$) (23). This study illustrates that social cognitive theory based weight loss interventions can be successful in an online format.

A study conducted by Cowan et al. sought to quantify the presence of health behavior theory constructs in iPhone apps that target physical activity. The study design was a content analysis of health behavior theory within iPhone applications used to target physical activity with the App Store's Health & Fitness category (37). The researchers returned an initial 1,336 possible apps but after examining the apps for exclusion criteria the researchers evaluated 127 total applications. The coders downloaded the apps, explored all functions, and then used a theory-based instrument to do content analysis of each application for the top four behavior theories (Health Belief Model, Transtheoretical Model, Theory of Planned Behavior, and Social Cognitive Theory) (37). Social cognitive

theory accounted for 20.38% ± 3.40 (mean ± SD) of the behavior constructs in the top 10% of applications (top 10% is based on the total theory score) (37). The Health Belief Model had the highest percentage in the top 10% of applications with 32.00% ± 4.54 (mean ± SD) (37). The authors of this study concluded that the iPhone apps included very few behavior change constructs (37). The authors also indicated that there is an available opportunity for health professionals to partner with app developers to incorporate behavior change constructs into the iPhone apps (37). The available research suggests that a weight loss intervention that has social cognitive theory incorporated into the program increases participants success, and incorporating these constructs into the iPhone applications would allow for greater success when using an app for weight loss (22, 23).

CHAPTER 3

METHODS

Participants

Thirty healthy individuals with no unresolved medical conditions or recent changes in prescription medications, who were between the ages of 19 and 58 years of age, owned an iPhone, had a BMI > 24 kg/ m^2, and desired to lose weight were recruited for this study. There were 130 responses to the study ad through Survey Monkey (see Appendix D for advertisement). Of those 130 responses, 46 individuals did not qualify from the initial screening criteria. The remaining 84 individuals were sent an email asking them to schedule an in-person visit for the final screening. Of the 84 individuals asked to participate, 36 were screened. Six individuals did not meet the study criteria. Participants were willing to track food intake daily for an eight week period via an app on their iPhone and travel to the downtown campus of Arizona State University on four separate occasions. The four research visits included the consenting visit prior to the start of the study and visits at study weeks 0, 4, and 8. The exclusion criteria included the use of any medications that affected weight status in the past three months, adherence to a weight loss diet plan within the past 3 months, and an unwillingness to provide a blood sample in a fasted state on three occasions (see Appendix E for Health History Questionnaire). All subjects provided written and informed consent prior to participation in the study (see Appendix A), and Arizona State University Institutional Review Board approved this research prior to initiation of recruitment (see Appendix B & C).

Study Protocol

Eligible participants were randomized into three smartphone app groups. The feedback control (MP-C) participants (n=10) used the diet tracking smartphone app “My Daily Plate” that encourages users to record foods consumed each day to meet the USDA's MyPlate food recommendation guidelines (http://cookingdistrict.com/mydailyplate 'GigaChef, LLC'). The picture (PIC-C) participants (n=10) tracked their daily dietary intake by taking pictures of all foods and beverages ingested each day using the MyDietitian app (http://www.mydietitian.com 'MyDietitian, LLC'). Body weight and physical activity is also tracked by the participant via a separate section of each app. The participant entered a daily weight from an at home scale to track weight change over time. The physical activity type and frequency is entered by the participant, and can be tracked over time within the app. While useful for the participant, body weight and physical activity was not tracked through the application by the researchers; rather, these factors were quantified at the test facility during study visits. Educational videos were available to view within the MyDietitian app if the user chose to view them. The picture plus registered dietitian (PIC-RD) participants (n=10) used the MyDietitian app described in the PIC-C group but also received daily feedback from a registered dietitian that specifically addressed the individual's daily dietary intake and eating patterns. The registered dietitans provided feedback based on the participants’ uploaded images of their meals, and were not provided any additional information about the participants’ goals regarding weight loss. The registered dietitians were trained to use their professional judgement when deciding how best to counsel the participants on their diet, and were not given specific guidelines for feedback. The participants did not have a direct link to communicate with the dietitans, although the participant was able to use the

description box feature on the app to provide more information about meals consumed. This feedback was in the form of a 3-4 sentence text message. The app has the capability to send the user a short video message, but this feature was not utilized during this study. The participants received only text feedback. All participants received generalized instructions on their respective iPhone app (see Appendix H, I, & J). For two consecutive days on three occasions during the trial (trial week 1, 4, and 8) participants were asked to log onto the ASA24 web site operated by the National Cancer Institute to record dietary intakes. The website provides data entry instructions to the user as the website is being used, no additional instructions were provided to the participants. At each study visit, participants were weighed on a calibrated Tanita scale (model #TBF300A, Tanita Corp. Arlington Hts, IL) and height (visit 1 only), waist circumference, and hip circumference were recorded. The participants' blood pressures were also taken at each visit with a Medline blood pressure cuff (model #MDS2001, Mundelein, IL) . For this measure, the participants' upper arm was bare and supported, and the cuff was placed approximately one inch above the bend in the elbow. All participants were sitting in a chair with their back supported, legs uncrossed, and feet on the floor. A fasting blood sample (2 TBLS) was collected on three occasions (trial visits 2, 3 & 4) and analyzed for bio-markers of health including fasting blood glucose, insulin, blood cholesterol, lipid panel (total triglycerides, HDL, CRP (a measure of inflammation in the body)), and A1c (a diabetes risk assessment measure) (see appendices N-K). Blood samples were collected from serum and plasma in a red top tube (no additive), EDTA, and Na Fluoride vacutainer tubes. A1c was analyzed on whole blood from the EDTA sample. Samples were spun at 3000rpm for fifteen minutes and plasma and serum aliquots were saved in microfuge

tubes and frozen at -80 degrees until analysis. Insulin was measured on serum. Lipids and CRP were measured on EDTA plasma, and glucose was measured on Na Fluoride plasma. Participants were also asked to fill out a validated physical activity questionnaire (38) on trial weeks 1, 4, and 8 (Appendix F). All participants that completed the trial filled out an exit survey on trial week 8 (Appendix G). Participants were also asked to fill out a physical activity questionnaire on trial weeks 1, 4, and 8 (Appendix F). All participants that completed the trial filled out an exit survey on trial week 8 (Appendix G).

The participants' compliance was determined by the number of days completed. The PIC-C and PIC-RD groups were considered compliant for the day if the participant uploaded at least one picture onto the MyDietitian app. The MP-C group was considered compliant if they emailed a picture of their MyPlate at the end of the day to the researcher. Adherence to protocol was encouraged by sending all participants weekly emails encouraging them to continue with the study and thanking them for their participation. The participants also received a $15 Target gift card at the fourth-week visit and at the eight-week visit to encourage attendance at follow-up visits during the trial (See Appendix R for detailed timeline).

Statistical Analysis

All data were analyzed using The Statistical Package for the Social Sciences (SPSS 18.0 for Windows, SPSS Inc., Chicago, IL). Mean, standard deviation, and range of data are reported using descriptive statistics. Data were checked for normality and transformed if needed to achieve normality. Repeated measures ANOVA was used to examine difference between groups over time. Due to a small sample size the non-

parametric Kruskal-Wallis Test was used to assess if there was a significant mean difference between groups over time. Baseline data by group were assessed using Oneway ANOVA; nominal data by group were assessed using Pearson's Chi Square analysis.

CHAPTER 4

RESULTS

Thirty participants with iPhone smartphones were recruited into this study to determine if feedback from a registered dietitian while using an iPhone app for dietary tracking would improve weight, biomarkers of health, and adherence to dietary tracking (PIC-RD) when compared to participants tracking with pictures alone (PIC-C) or an iPhone app designed to mimic the USDA's MyPlate (MP-C). Thirty participants began the trial and during the trial six participants declined to continue to participate, leaving twenty-four participants that completed the eight week trial. Five of the six participants felt it was too time consuming to continue to meet on campus for follow-up appointments and one subject switched phone plans and no longer owned an iPhone. All subjects were recruited via an online survey with distribution through the Arizona State University list-serves. After completing an initial screening the participants were randomized into one of three groups; MP-C (n=10), PIC-C (n=10), and PIC-RD (n=10). There was no significant mean difference in demographics (Table 1) or anthropometric characteristics (Table 2) between the groups.

Table 1: Demographic Data

	MP-C (n=10)	PIC-C (n=10)	PIC-RD (n=10)	P-Value**
Age	27.1 +/- 6.0	28 +/- 8.9	28.8 +/- 11.3	0.602
Gender				
Male	3	3	3	1.000
Female	7	7	7	
Education				
Some College	4	6	7	0.387
College Graduate	6	4	3	
Ethnicity				
Hispanic or Latino	1	3	2	0.535
Not Hispanic or Latino	9	7	8	
Race				
American Indian/Alaskan Native	1	0	0	
African American	2	0	0	0.243
White	7	9	9	
Asian	0	1	0	
Other	0	0	1	
Smoker				
Yes	0	0	1	0.501
No	7	9	7	
Previously smoked, but quit	3	1	2	
Medications				
Yes	3	3	2	0.843
No	7	7	8	

**Pearson's Chi Square

Table 2: Anthropometric Characteristics Initial Screening

	MP-C (n=10)	PIC-C (n=10)	PIC-RD (n=10)	P-Value**
Waist (in)	38.3 +/- 7.2	39.7 +/- 7.4	38.6 +/- 4.2	0.871
Weight (lbs)	195.5 +/- 48.6	197.5 +/- 62.2	195.4 +/- 29.4	0.994
Hip (in)	46.1 +/- 7.6	45.8 +/- 7.0	45.6 +/- 2.3	0.984
Systolic Blood Pressure	123.6 +/- 17.4	122.5 +/- 13.7	122.6 +/- 9.4	0.981
Diastolic Blood Pressure	76.7 +/- 9.7	84.2 +/- 10.7	79.1 +/- 9.1	0.236
Body Mass Index	31.4 +/- 7.3	32.5 +/- 8.1	30.3 +/- 3.4	0.754
Body Fat (% weight)	34.4 +/- 9.1	36.9 +/- 5.9	36.9 +/- 6.7	0.683
Exercise (MET)	26.7 +/- 18.3	31.7 +/- 31.0	28.0 +/- 19.6	0.887

**One-Way ANOVA

There was no significant difference in attrition rates between groups. 100% of the MP-C group, 70% of the PIC-C group, and 70% of the PIC-RD completed the eight week study. There was an overall completion rate of 80%. There was no significant mean difference in anthropometric characteristics between groups at the completion of the eight week trial (Table 3). At the completion of the 8-week trial, body weight did not change among groups ($p=0.896$) or over time ($p=0.998$) (Figure 1). Similarly, body fat did not change among groups ($p=0.298$), but body fat tended to increase in all participants over time $p=0.055$) (Figure 2). Waist and hip circumferences did not change among groups; however, both measures decreased significantly over time in all participants ($p<0.001$).

Table 3: Anthropometric Characteristics Before and After Treatment

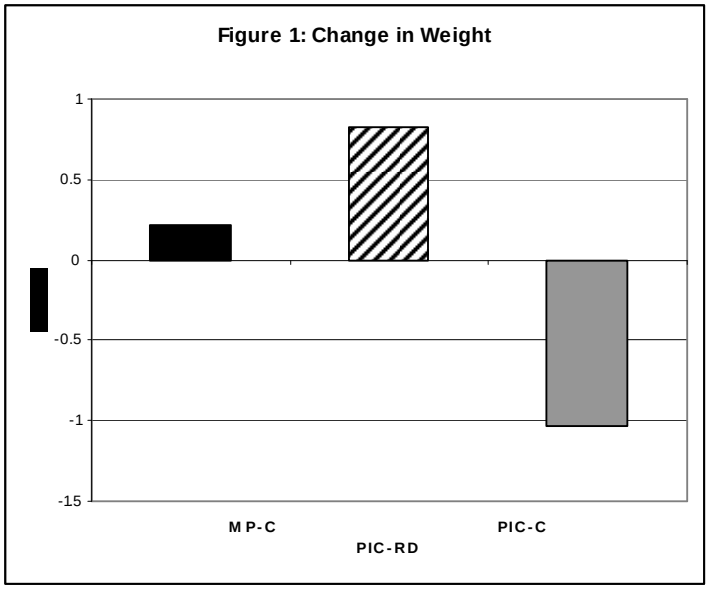
	MP-C (n=10)	PIC-C (n=7)	PIC-RD (n=7)	P-Value** Group Interaction	P-Value*** Time
Waist (in)	Pre: 38.3 +/- 6.7 Post: 37.2 +/- 6.4 Median Δ: -1.25	Pre: 41.8 +/- 9.7 Post: 40.2 +/- 10.8 Median Δ: -1.50	Pre: 37.7 +/- 4.6 Post: 36.9 +/-5.0 Median Δ: -0.50	0.529	<0.001
Weight (lbs)	Pre: 195.8 +/- 48.6 Post: 196.0 +/- 47.3 Median Δ: 0.10	Pre: 206.5 +/- 70.3 Post: 207.3 +/- 71.9 Median Δ: 0.80	Pre: 194.8 +/- 28.5 Post: 193.8 +/- 27.8 Median Δ: 0.80	0.896	0.998
Hip (in)	Pre: 46.4 +/- 7.2 Post: 45.0 +/- 6.7 Median Δ: -1.75	Pre: 45.9 +/- 7.4 Post: 44.7 +/- 8.2 Median Δ: -1.5	Pre: 45.6 +/- 2.1 Post: 44.2 +/- 2.1 Median Δ: -0.50	0.864	<0.001
Systolic Blood Pressure	Pre:112.2 +/- 13.1 Post: 115.7 +/- 10.1 Median Δ: 5.00	Pre: 125.1 +/- 19.3 Post: 123.9 +/- 17.2 Median Δ: -3.00	Pre: 109.9 +/- 5.3 Post: 113.4 +/- 7.5 Median Δ: 3.00	0.391	0.419
Diastolic Blood Pressure	Pre: 71.1 +/- 8.8 Post: 72.7 +/- 9.3 Median Δ: 1.50	Pre: 84.3 +/- 14.6 Post: 84.4 +/- 13.2 Median Δ: 0.00	Pre: 74.4 +/- 5.1 Post: 77.7 +/- 11.8 Median Δ: -2.00	0.904	0.735
Body Mass Index	Pre: 31.4 +/- 7.2 Post: 31.5 +/- 7.1 Median Δ: 0.00	Pre: 33.6 +/- 9.3 Post: 33.7 +/- 9.5 Median Δ: 0.10	Pre: 29.4 +/- 3.2 Post: 29.3 +/- 3.0 Median Δ: 0.10	0.921	0.910
Body Fat (% weight)	Pre: 35.5 +/- 9.5 Post: 36.8 +/- 8.4 Median Δ: 1.60	Pre: 37.5 +/- 7.0 Post: 39.3 +/- 7.4 Median Δ: 0.40	Pre: 35.9 +/- 6.8 Post: 35.8 +/- 6.2 Median Δ: -0.10	0.298	0.055
Exercise (MET)	Pre: 29.7 +/- 21.5 Post: 33.3 +/- 27.4 Median Δ: 3.50	Pre: 41.7 +/- 42.0 Post: 39.7 +/- 36.3 Median Δ: -3.00	Pre: 25.9 +/- 15.7 Post: 33.6 +/- 33.0 Median Δ: 0.00	0.833	0.340
Adherence %	69.3 +/- 23.5	53.0 +/- 27.5	68.3 +/- 38.1	0.47	

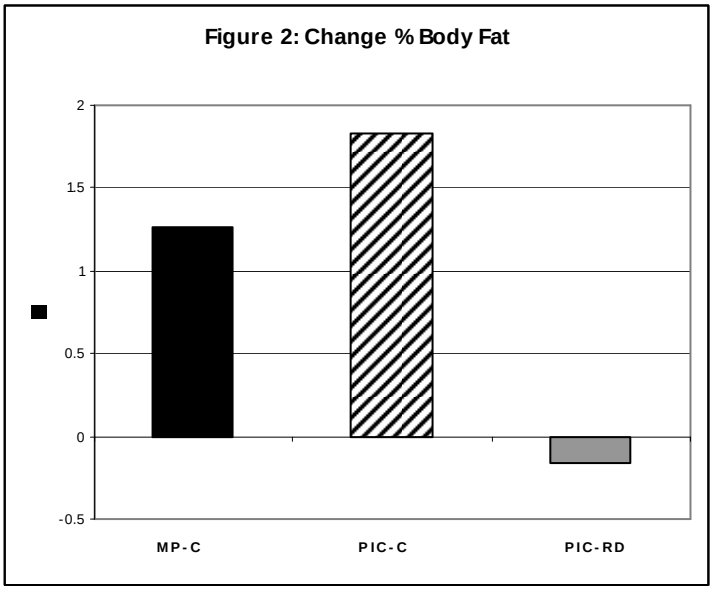
There was a significant mean difference from initial screening in SBP ($p<0.001$) and %Fat ($p<0.001$) using a paired sample t-test, there was no significant difference between groups.

MET=standard metabolic equivalent unit used to estimate the amount of oxygen used by the body during physical activity.

**Kruskal-Wallis Test

***Repeated Measures ANOVA





There was no significant change in blood biomarkers between groups at the completion of the eight week trial (Table 4). However, HDL cholesterol rose significantly (+6%), hemoglobin A1c fell significantly (-3%) in the participants overall during the trial ($P<0.05$; Table 4), and there was a trend towards significance between groups for glucose ($p=0.084$).

Table 4: Blood Biomarkers of Health Before and After Treatment

	MP-C (n=10)	PIC-C (n=7)	PIC-RD (n=6)	P-Value** Group Interaction	P-Value *** Time
Cholesterol (mg/dL)	Pre: 173.8 +/- 35.7 Post: 176.3 +/- 39.3 Median Δ: 4.25	Pre: 163.6 +/- 35.9 Post: 167.6 +/- 28.0 Median Δ: 2.50	Pre: 158.4 +/- 21.6 Post: 162.0 +/- 32.3 Median Δ: -0.50	0.973	0.577
HDL (mg/dL)	Pre: 53.8 +/- 19.0 Post: 57.4 +/- 17.4 Median Δ: 1.5	Pre: 42.4 +/- 12.8 Post: 45.9 +/- 16.9 Median Δ: 3.50	Pre: 48.8 +/- 7.9 Post: 50.6 +/- 11.6 Median Δ: 1.75	0.813	0.012
Triglyceride (mg/dL)	Pre: 122.1 +/- 59.3 Post: 97.2 +/- 51.5 Median Δ: -14.91	Pre: 123.7 +/- 79.0 Post: 130.0 +/- 66.5 Median Δ: 6.73	Pre: 93.7 +/- 30.7 Post: 96.6 +/- 29.2 Median Δ: 1.14	0.179	0.820
LDLc (mg/dL)	Pre: 95.6 +/- 29.5 Post: 99.5 +/- 35.0 Median Δ: 3.37	Pre: 96.5 +/- 32.0 Post: 95.7 +/- 25.6 Median Δ: -5.61	Pre: 90.8 +/- 13.9 Post: 92.1 +/- 19.4 Median Δ: -2.50	0.768	0.516
CRP (mg/L)	Pre: 4.5 +/- 5.7 Post: 3.8 +/- 4.4 Median Δ: -0.18	Pre: 4.6 +/- 4.7 Post: 4.8 +/- 5.1 Median Δ: 1.40	Pre: 7.2 +/- 8.8 Post: 5.9 +/- 7.5 Median Δ: -0.82	0.358	0.697
Glucose (mg/dL)	Pre: 92.1 +/- 5.4 Post: 94.0 +/- 7.1 Median Δ: 2.50	Pre: 100.9 +/- 13.8 Post: 101.2 +/- 14.9 Median Δ: 2.00	Pre: 88.1 +/- 3.6 Post: 86.3 +/- 4.1 Median Δ: -1.50	0.084	0.930
Insulin (uU/ml)	Pre: 14.2 +/- 8.0 Post: 13.4 +/- 6.7 Median Δ: 0.34	Pre: 24.0 +/- 22.4 Post: 24.1 +/- 22.4 Median Δ: -1.34	Pre: 14.4 +/- 5.8 Post: 15.1 +/- 5.7 Median Δ: 0.78	0.685	0.895
HOMA-IR	Pre: 3.3 +/- 2.1 Post: 3.2 +/- 1.8 Median Δ: 0.13	Pre: 6.6 +/- 7.5 Post: 6.5 +/- 7.5 Median Δ: -0.08	Pre: 3.2 +/- 1.4 Post: 3.3 +/- 1.4 Median Δ: 0.12	0.864	0.924
A1c	Pre: 5.1 +/- 0.3 Post: 5.0 +/- 0.3 Median Δ: -0.15	Pre: 5.4 +/- 0.4 Post: 5.2 +/- 0.4 Median Δ: -0.20	Pre: 5.0 +/- 0.2 Post: 5.0 +/- 0.3 Median Δ: -0.10	0.736	0.002

There was no significant mean difference between groups at baseline. One participant in the PIC-RD group was unable to give blood.

HOMA-IR=Homeostasis model assessment-estimated insulin resistance ((fasting plasma insulin x fasting plasma glucose)/ 22.5)

LDLc=total cholesterol – (HDL + Triglycerides/5)

**Kruskal-Wallis Test

***Repeated Measures ANOVA

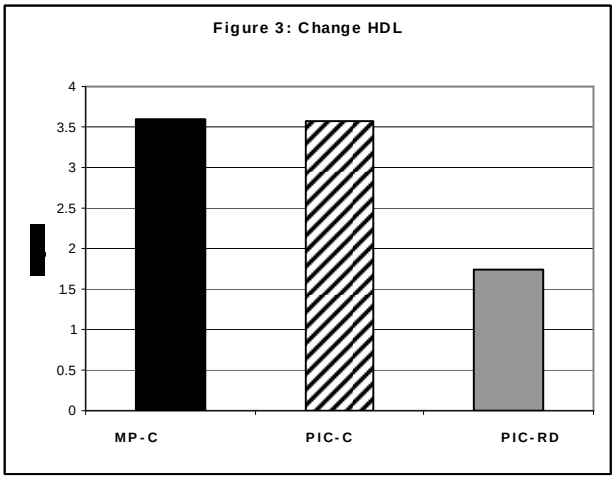


Figure 3: Change HDL
4
3.5
3
2.5
2
1.5
1
0.5
0
MP-C
PIC-C
PIC-RD

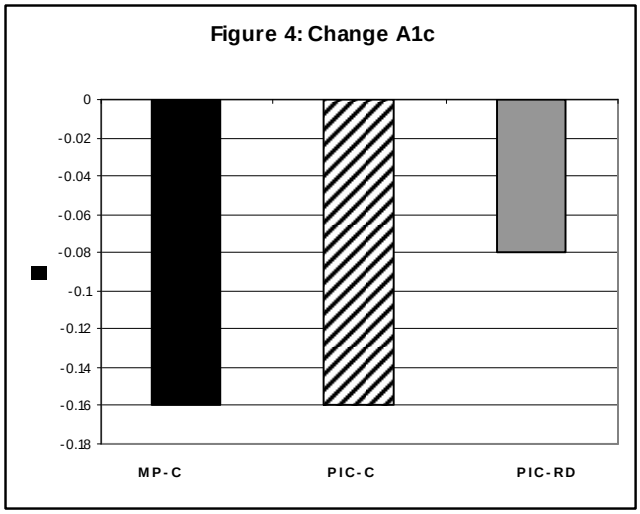


Figure 4: Change A1c
0
-0.02
-0.04
-0.06
-0.08
-0.1
-0.12
-0.14
-0.16
-0.18
MP-C
PIC-C
PIC-RD

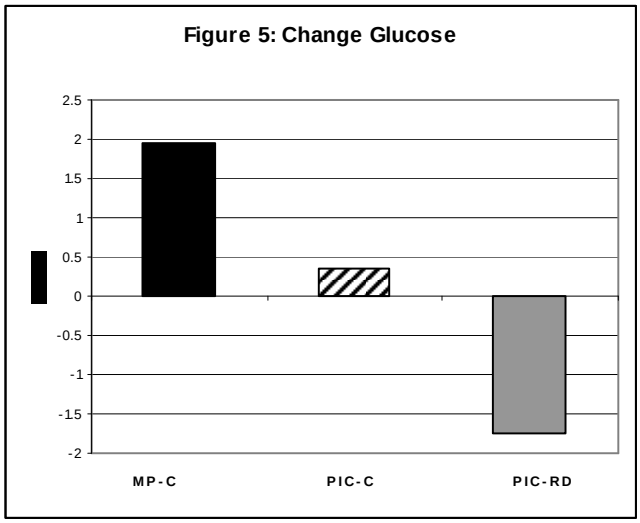


Figure 5: Change Glucose
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
MP-C
PIC-C
PIC-RD

An exit survey was given at the completion of the eight week trial. The exit survey consisted of seven statements. Each statement required the participant to mark on a line showing how strongly they agreed or disagreed with the statement listed. The higher the score, the more the participant agreed with the statement listed (Table 5). There was a significant mean difference between the MP-C and PIC-RD group for statement 1 (p=0.018). The statement was "Using the iPhone app was helpful in keeping me on track toward my weight loss goal". The PIC-RD group agreed with this statement significantly more than the MP-C group (p=0.018).

Table 5: Exit Survey

	MP-C (n=10)	**PIC-C (n=7)**	**PIC-RD (n=7)**	**P-Value***
1. Using the iPhone app was helpful in keeping me on track toward my weight loss goal.				
	-1.45 +/- 5.16	1.58 +/- 2.86	3.23 +/- 0.06	0.049**
2. The app I used for recording my daily food intake was too time consuming to be practical.				
	-1.85 +/- 4.64	-2.39 +/- 2.85	-1.45 +/- 4.66	0.917
3. I was more aware of my eating habits because I was recording my food intake.				
	3.34 +/- 3.21	1.86 +/- 2.61	4.51 +/- 4.25	0.359
4. I am more confident in my ability to lose weight after participating in this study.				
	-1.07 +/- 3.59	0.80 +/- 3.40	2.19 +/- 2.82	0.156
5. I have learned much about my food habits and my eating has improved by participating in this study.				
	1.53 +/- 3.49	0.14 +/- 4.17	2.94 +/- 2.66	0.344
6. I will continue to record my food intake after the study is over.				
	1.74 +/- 4.20	0.66 +/- 3.50	0.18 +/- 4.01	0.709
7. Having a dietitian providing feedback to my diet on a daily basis was (or would have been) beneficial.				
	4.06 +/- 2.51	3.33 +/- 3.25	5.09 +/- 3.15	0.540

*One-Way ANOVA

** Significant mean difference between MP-C and PIC-RD group (p=0.018, LSD post-hoc)

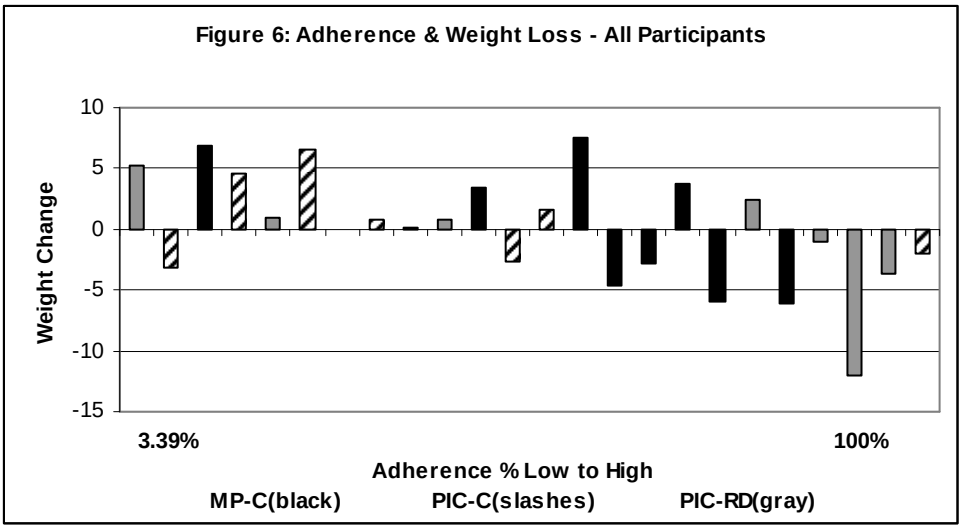
Participants' diets were analyzed over the eight week period using 24-hour diet recalls that were entered into the ASA24 database at baseline, 4 weeks, and 8 weeks. The participants in all groups significantly reduced their mean energy intake from baseline at

the completion of the 8 week trial (p=0.022). There was no significant change in the other measures of diet quality over the eight week period or between groups (Table 6).

Table 6: Diet Quality

	MP-C 0 (n=6) 4 (n=6) 8 (n=4)	**PIC-C** 0 (n=4) 4 (n=4) 8 (n=3)	**PIC-RD** 0 (n=3) 4 (n=3) 8 (n=2)	**P-Value*** Time	**P-Value*** Group Interaction
Energy(kcal)					
0 Weeks	1654.7+/-903.4	2084.2+/-312.6	2616.1 +/- 1200.4	0.022	0.865
4 Weeks	1390.7+/-490.9	1550.1+/-391.7	1746.3 +/- 604.9		
8 Weeks	1033.8+/-398.5	1541.4+/-295.1	2025.7 +/- 84.8		
Fat%energy					
0 Weeks	30.6 +/- 8.1	33.5 +/- 9.7	38.8 +/- 2.4	0.715	0.743
4 Weeks	29.2 +/- 16.0	40.4 +/- 9.7	41.1 +/- 7.1		
8 Weeks	26 +/- 14.7	31.6 +/- 3.5	46.8 +/- 14.4		
Vitamin C (mg/1000kcal)					
0 Weeks	36.2 +/- 49.9	37.3 +/- 20.4	23.0 +/- 23.9	0.625	0.900
4 Weeks	63.26 +/- 101.0	29.7 +/- 15.4	41.8 +/- 14.4		
8 Weeks	72.0 +/- 75.6	18.5 +/- 22.4	25.8 +/- 11.6		
Fiber (g/1000kcals)					
0 Weeks	12.7 +/- 8.9	10.1 +/- 2.5	7.77 +/- 2.1	0.311	0.962
4 Weeks	16.4 +/- 18.4	7.6 +/- 1.0	9.20 +/- 2.7		
8 Weeks	11.3 +/- 4.2	7.3 +/- 1.0	5.86 +/- 3.0		
Sodium (mg/1000kcal)					
0 Weeks	1814.4 +/- 494.7	1635.5 +/- 224.3	1835.9 +/- 403.2	0.421	0.486
4 Weeks	2286.3 +/- 748.5	2115.7 +/- 690.4	1718.5 +/- 309.5		
8 Weeks	1887.2 +/- 141.8	1697.7 +/- 367.3	2257.8 +/- 349.6		
Sugar (g/1000kcal)					
0 Weeks	47.2 +/- 27.1	52.0 +/- 25.4	31.4 +/- 13.9	0.996	0.839
4 Weeks	59.1 +/- 52.1	40.1 +/- 21.3	29.6 +/- 17.6		
8 Weeks	41.0 +/- 14.9	54.4 +/- 23.9	35.2 +/- 26.9		

*Two-Way ANOVA



Adherence to self-monitoring was assessed at the completion of the trial. The researchers obtained a daily upload log from the MyDietitian app developers. The participants in these groups were considered adherent for the day if they uploaded at least once during the day. The MP-C group was asked to email a picture of their MyPlate at the end of each day. The MP-C group participants were considered adherent for the day if they sent an email containing a picture of their MyPlate. Adherence to the phone app explained 29% of the variation in all of the study participants' weight change, and there was a significant correlation between % adherence and weight change when all groups were combined ($r=0.536$, $p=0.007$). There was not a significant difference in adherence between groups ($p=0.467$). The correlation between % adherence and weight change shows a trend towards significance in the feedback control group ($p=0.095$) and the PIC-RD group (0.110).

CHAPTER 5

DISCUSSION

Widely marketed techniques for weight management have been embraced by the general public. It is currently estimated that in 2013 the United States weight loss market will reach a value of $66.5 billion per year (39). While the weight loss market is largely composed of weight loss supplements, food products, diet drugs, and diet programs; market researchers anticipate more technology-based weight loss programs to appear in the 2013 market (39). In 2012, Flurry reported that there are 165 million active Android and Apple devices in the United States and that these devices are used by 78% of the adult population ages 15-64 (40). These mobile devices are now being used to target weight loss. It is estimated that by 2017, the mobile health app market will be worth upwards of $26 billion dollars (41). Currently there are more than 97,000 mobile apps available related to health and fitness (41). While this market is becoming very profitable, there is very little research published on the value of smartphone apps for weight loss. At this time, there is no available published research on the use of a smartphone app with feedback from a registered dietitian for dietary intake tracking and weight loss.

This research compared the use of a new dietary self-monitoring smartphone app (MyDietitian) with and without the feedback of a registered dietitian to a feedback control app that encouraged adherence to the USDA's MyPlate dietary guidelines. We hypothesized that the use of the smartphone app with feedback from a registered dietitian would provide a significant improvement in weight loss and bio-markers of health when compared to the non-feedback and feedback control app groups. We also hypothesized that the use of the smartphone application with feedback from a registered dietitian would

improve adherence to dietary tracking when compared to the non-feedback and feedback control group. Finally, we hypothesized that the use of the smartphone application with feedback from a registered dietitian would improve diet quality when compared to the non-feedback and feedback control group.

All study participants had a desire to lose weight and had a BMI of at least 24 kg/m^2. The participants were not provided advanced training on their assigned phone application. The study was designed to mimic free-living individuals that downloaded apps for personal use. There was no significant difference in demographic or anthropometric characteristics between groups. All participants were sent weekly emails thanking them for their participation in the study.

All participants were assessed for anthropometric characteristics and blood bio-markers of health on three separate occasions (week 0, 4, 8). All of the groups significantly reduced their waist and hip circumference ($p<0.001$) at the completion of the trial. All of the groups significantly increased HDL levels ($p=0.012$), decreased A1C levels ($p=0.002$), and decreased 24-h calorie intakes at the completion of the trial. However, there were no significant differences between groups in any of the anthropometric markers or blood bio-markers of health.

The results indicate that while significance was not found the PIC-RD group was the only diet group to lose weight. A study conducted with a larger sample size may produce significant results. A power calculation based on data derived from this investigation indicates that ~17 participants per group are necessary to observe a 4lb weight change between groups over 8 weeks at 80% power. However, 250 participants

per groups are necessary if the change in weight is only expected to be 1 pound between groups.

The energy intake and diet quality of the participant were analyzed over the eight week period by having the participants enter 24-hour diet recalls into the ASA24 database at baseline, 4 weeks, and 8 weeks. Overall, participants significantly reduced their mean energy intake at trial week 8 versus baseline ($p=0.022$). There was no significant change in measures of diet quality (vitamin C, fiber, sodium, and sugar) over the eight week period or between groups. The lack of change in diet quality may be due to the low number of complete 24-hour diet recalls. At baseline only 60% of the MP-C, 40% of the PIC-C, and 30% of the PIC-RD group participants completed the 24-hour diet recalls. At the completion of the study 40% of the MP-C, 30% of the PIC-C, and 20% of the PIC-RD group participants completed the 24-hour diet recalls..

At the completion of the study the participants were asked to complete an exit survey. There was a significant mean difference between the MP-C and PIC-RD group for statement 1 ($p=0.018$). The statement was “Using the iPhone app was helpful in keeping me on track toward my weight loss goal”. The PIC-RD group agreed with this statement significantly more than the MP-C group ($p=0.018$). This is most likely due to the differences in the iPhone apps themselves. It is likely that the MyDietitian app was more user friendly and had more useful features which gave the users the perception that the app was more helpful.

Adherence to self-monitoring for the PIC-C and PIC-RD groups were assessed at the completion of the trial. The researchers obtained a daily upload log from the MyDietitian application developers. The participants in these groups were considered

adherent for the day if they uploaded at least once in the day assessed. The MP-C group was asked to email a picture of their MyPlate at the end of each day. The MP-C group participants were considered adherent for the day if they sent an email containing a picture of their MyPlate. Adherence was 69.33% ±23.50%, 68.27% ±38.08, and 52.97% ±27.50% for the MP-C, PIC-RD, and PIC-C groups respectively. There was no significant difference in adherence between groups ($p=0.467$).

Adherence to self-monitoring was further assessed by separating participants into two groups based on the criteria of having less than 50% or greater than 50% adherence to self-monitoring. A significant difference in weight loss was found between the high and low adherence groups ($p=0.046$). The group that adhered to self-monitoring greater than 50% of the time had an average weight loss of 1.7 pounds while the group that adhered to self-monitoring less than 50% of the time had an average weight gain of 2.7 pounds. These results were supported by a previous study conducted by Kong et al.. The study conducted by Kong et al. found that women who were in the 75^{th} percentile for food journaling had a 3.7% greater weight loss than the women in the 25^{th} percentile for food journaling (3). The women in the 75^{th} percentile also had a 2.7% greater weight loss than the women who did not comply with food journaling (3). These results are also consistent with research conducted with the National Weight Control Registry. The NWCR identified that a key component of successful weight loss and long-term weight maintenance was consistent self-monitoring and recording of dietary intake (27).

Adherence to self-monitoring as a weight loss tool has been widely supported by previous studies (3, 4, 6, 9). The significant difference in weight loss found between the low and high adherence groups (without a significant difference between phone

application groups) indicates that regardless of the phone application used, adherence to self-monitoring is the key for successful weight loss.

The Cochran Collaboration's review article published in 2012 found that while a computer-based intervention was superior to a minimal intervention, the in-person treatment group was superior to both the minimal and the computer-based intervention groups (28). A feasibility study conducted by Campbell et al. found that diet and lifestyle interventions led by registered dietitians could be a feasible strategy to improve outcomes in overweight and obese cancer patients (29). The participants in this study received 16 group-based sessions led by registered dietitians (29). The Diabetes Prevention Program also found that dietitians can play a pivotal role in lifestyle interventions (30). While the results from these studies indicate that interventions conducted by registered dietitians are effective they also indicate that the level of feedback provided by the MyDietitian application may have been lacking. Both the Campbell study and the Diabetes Prevention Program utilized a 16 session lifestyle change curriculum (29,30). The Diabetes Prevention Program also used individual sessions once per month after the initial 16 sessions were completed (30). The individual sessions allowed the dietitians to tailor the intervention to the participant, and allowed them to utilize their counseling skills to build a bond with the person (30). While the MyDietitian application provided feedback to the PIC-RD participants, feedback was 3-4 sentences per day only and participants were not allowed to actively interact with their dietitian which may have reduced the ability for the dietitian to build a bond with the participant.

The study conducted by Collins et al. also indicated that the features available to the PIC-RD group could be further enhanced to allow for greater adherence and weight

loss. Collins et al. developed a web-based intervention based on social cognitive theory of behavior change (23). This intervention included access to the following features for the basic group: individualized daily calorie targets, online food and exercise diaries, weekly menu plans with a grocery list, weekly educational tips and challenges, online forums for social support, daily and weekly calculations of nutrition summaries and energy balance, weekly newsletters, self-monitoring of body weight and waist circumference reminders, goal-setting options with graphical display (23). The enhanced group had access to all the basic features plus these additional features: a personalized enrollment report which suggests weight loss goals and key behaviors the participant will need to change to reach the goals, weekly automated personal feedback for nutrition and exercise levels based on the participants entries into the online diary, weekly feedback on their current level of weight loss success, and a reminder schedule for diary compliance (23). Collins et al. found significant between-group differences in the percentage of participants who lost greater than or equal to 10% of their baseline weight (control: 0%, basic: 18%, enhanced 28%; $p<0.001$), suggesting that the enhanced version of the web-site allowed participants to lose the greatest amount of weight (23) The MyDietitian app is lacking in the above listed online features, and including these features could increase the effectiveness of this application for weight loss and health.

Including features in iPhone apps that incorporate behavior change constructs may increase the effectiveness of iPhone apps and weight loss. Cowan et al. evaluated 127 total iPhone apps for the presence of health behavior theory constructs and found that very few iPhone apps included health behavior theory (37). The research available suggests that a weight loss intervention that includes social cognitive theory into the

program increases participants' success (22, 23). MyDietitian could benefit from incorporating health behavior theory into the application.

There were several limitations present in this study. The sample sizes were small which greatly reduced the power to observe significant changes in the outcome measures. Adherence to protocol was low reducing the internal validity; however, the study was designed to mimic natural use of the apps. The information provided by the registered dietitians to the PIC-RD participants may not have been consistent in that group. Each PIC-RD participant was randomly assigned to a registered dietitian and multiple dietitians were used. The level of counseling and type of nutritional information provided to each of the participants likely varied between individuals.

The participants were recruited from the Arizona State University list-serves which created a level of homogeneity that would not exist in a larger sample from the general population. The study also does not address individuals that own a smartphone brand other than an Apple iPhone. The study only included individuals from the southwest and cannot be generalized to the elderly, children, and individuals that live in other parts of the United States or worldwide.

CHAPTER 6

CONCLUSION

Our study utilized two different iPhone apps in three different groups to track dietary intake. Two groups used the MyDietitian iPhone app and one group used the MyPlate iPhone app. The purposes of this study was to compare the use of the new dietary self-monitoring iPhone app (MyDietitian) with and without the feedback of a registered dietitian against the feedback control app MyPlate to assess the differences in adherence, anthropometric characteristics, diet quality, and bio-markers of health between groups. All of the groups had a significant reduction in waist and hip circumference, a significant increase in HDL and a significant decrease in A1c, and a reduction in their calorie intake during the length of the study. There was no significant difference between groups. The individuals that lost the largest amount of weight were the individuals that had adherence to self-monitoring greater than 50% of study days. These results indicate that regardless of which iPhone app the participant is using, the more an individual uses self-monitoring the greater the successes in terms of weight loss. This study indicates that adherence to self-monitoring is the key for weight loss success. App developers should incorporate ways to increase self-monitoring adherence into weight loss phone apps. Future studies exploring ways to increases self-monitoring of diet within iPhone applications are needed.

CHAPTER 7

FUTURE DIRECTIONS

App developers should incorporate ways to increase self-monitoring adherence into weight loss phone apps. App developers could utilize smartphone features that allow the app to periodically remind the individual to record daily meals and snacks. The app could also reward the individual through a point system within the app when a defined level of adherence to dietary tracking is reached. Allowing the individual to self-monitor eating behaviors in a fun and engaging way may lead to more successful weight loss through smartphone apps. In the future, smartphone apps developed for weight loss may be built with social cognitive theory as a foundation providing individuals with a readily accessible weight management tool.

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APPENDIX A

CONSENT FORM

ASU School of Nutrition & Health Promotion
ARIZONA STATE UNIVERSITY

ASU IRB Approved
by Carol Johnston
9/13/12 to 9/12/13

ASU NUTRITION: iPhone apps and dieting success

INTRODUCTON
The purposes of this form are (1) to provide you with information that may affect your decision as to whether or not to participate in this research study, and (2) to record your consent if you choose to be involved in this study.

RESEARCHERS
Dr. Carol Johnston, ASU nutrition professor, and Claudia Thompson-Felty, graduate student, have requested your participation in a research study.

STUDY PURPOSE
The purpose of this research study is to evaluate the usefulness of iPhone apps as a tool to help individuals improve food choices, lose weight, and improve health markers in blood.

DESCRIPTION OF RESEARCH STUDY
Qualifying participants desire to lose weight and are willing to use their iPhones daily to record food intake for eight weeks. Participants will be randomly assigned to one of three smart phone apps which will be provided at no cost. You will be asked to record all food intakes on your iPhone daily, a strategy that has been shown in previous research to facilitate weight loss. Initially you will come to the test site to complete a brief health history questionnaire to demonstrate the absence of medical conditions or situations that may impact the study. At this visit you will be trained to complete 24-hour diet recalls on the web; these recalls will take place randomly during the 8-week trial. Your blood pressure, weight, and height will be measured, and we will measure your waist and hip circumferences. The scale that determines your body weight will also provide information regarding your body composition by sending a weak electrical current through your body that cannot be felt. You will be scheduled for study visits 2, 3, and 4 which correspond to the start, middle, and end of the 8-week trial. For these three visits, you will be asked to present in a fasted state (no food or drink [with the exception of water] for >10 hours). At each of these 3 visits, a blood sample (2 tablespoons) will be collected to analyze for common health markers including glucose and cholesterol. The same measurements will be performed as in visit#1. Each visit will last about 30 minutes. You will receive weekly emails from the researchers so any questions can be answered. There is a short exit survey we will ask you to complete at the end of the trial.

If you begin taking new medications during the study, you are to notify the study investigators. About 60 people will participate in this study. This study will take place at the ASU Downtown campus.

RISKS
There are no foreseeable risks associated with this study. Individuals may become bored or frustrated with the daily protocol of food entry on the iPhone. Blood draws may cause dizziness, nausea, and faintness; a trained phlebotomist will perform all blood draws and respond immediately to any adverse reaction.

BENEFITS
This study will provide information regarding the usefulness of iPhone apps for improving diet adherence and possibly weight loss. You may learn more about your diet if you participate in this study.

NEW INFORMATION
If the researchers find new information during the study that would reasonably change your decision about participating, then they will provide this information to you.

CONFIDENTIALITY
All information obtained in this study is strictly confidential unless law requires the disclosure. The results of this research study may be used in reports, presentations, and publications, but your name or identity will not be revealed. In order to maintain confidentiality of your records, Dr. Johnston will use subject codes on all data

collected, maintain a master list separate and secure from all data collected, and limit access to all confidential information to the study investigators.

WITHDRAWAL PRIVILEGE
You may withdraw from the study at any time for any reason without penalty or prejudice toward you. Your decision to withdraw would not affect you in any manner.

COSTS AND PAYMENTS
You will receive two $15.00 in gift certificates to Target if you participate in this study. The first gift card will be received at visit#2 ($15) and at visit#4 ($15).

COMPENSATION FOR ILLNESS AND INJURY
If you agree to participate in the study, then your consent does not waive any of your legal rights. However, in the event of harm, injury, or illness arising from this study, neither Arizona State University nor the researchers are able to give you any money, insurance coverage, free medical care, or any compensation for such injury. Major injury is not likely but if necessary, a call to 911 will be placed.

VOLUNTARY CONSENT
Any questions you have concerning the research study or your participation in the study, before or after your consent, can be answered by Dr. Carol Johnston (602-827-2265).

If you have questions about your rights as a subject/participant in this research, or if you feel you have been placed at risk, you can contact the Chair of the Human Subjects Institutional Review Board, through the ASU Office of Research Integrity and Assurance, at 480-965 6788.

This form explains the nature, demands, benefits and any risk of the project. By signing this form you agree knowingly to assume any risks involved. Remember, your participation is voluntary. You may choose not to participate or to withdraw your consent and discontinue participation at any time without penalty or loss of benefit. In signing this consent form, you are not waiving any legal claims, rights, or remedies. A copy of this consent form will be given to you.

Your signature below indicates that you consent to participate in the above study.

_____ _____
Subject's Signature Printed Name

_____
Date

_____ _____
Contact phone number Email (print clearly)

INVESTIGATOR'S STATEMENT
"I certify that I have explained to the above individual the nature and purpose, the potential benefits, and possible risks associated with participation in this research study, have answered any questions that have been raised, and have witnessed the above signature. These elements of Informed Consent conform to the Assurance given by Arizona State University to the Office for Human Research Protections to protect the rights of human subjects. I have provided the subject/participant a copy of this signed consent document."

Signature of Investigator_____ Date_____

ASU IRB Approved
by Carol Johnston
9/13/12 to 9/12/13

APPENDIX B

IRB APPROVAL

ASU Knowledge Enterprise Development

Office of Research Integrity and Assurance

To:	Carol Johnston ABC 132
From:	Carol Johnston, Chair Biosci IRB
Date:	09/13/2012
Committee Action:	**Expedited Approval**
Approval Date:	09/13/2012
Review Type:	Expedited F2 F4 F7
IRB Protocol #:	1209008222
Study Title:	iPhone Apps and Dieting Success
Expiration Date:	09/12/2013

The above-referenced protocol was approved following expedited review by the Institutional Review Board.

It is the Principal Investigator's responsibility to obtain review and continued approval before the expiration date. You may not continue any research activity beyond the expiration date without approval by the Institutional Review Board.

Adverse Reactions: If any untoward incidents or severe reactions should develop as a result of this study, you are required to notify the Biosci IRB immediately. If necessary a member of the IRB will be assigned to look into the matter. If the problem is serious, approval may be withdrawn pending IRB review.

Amendments: If you wish to change any aspect of this study, such as the procedures, the consent forms, or the investigators, please communicate your requested changes to the Biosci IRB. The new procedure is not to be initiated until the IRB approval has been given.

Please retain a copy of this letter with your approved protocol.

APPENDIX C

AMENDMENT TO APPROVED PROTOCOL

ASU Knowledge Enterprise Development

Office of Research Integrity and Assurance

To:	Carol Johnston ABC 132
From:	Carol Johnston, Chair Biosci IRB
Date:	10/01/2012
Committee Action:	**Amendment to Approved Protocol**
Approval Date:	10/01/2012
Review Type:	Expedited F12
IRB Protocol #:	1209008222
Study Title:	iPhone Apps and Dieting Success
Expiration Date:	09/12/2013

The amendment to the above-referenced protocol has been APPROVED following Expedited Review by the Institutional Review Board. This approval does not replace any departmental or other approvals that may be required. It is the Principal Investigator's responsibility to obtain review and continued approval of ongoing research before the expiration noted above. Please allow sufficient time for reapproval. Research activity of any sort may not continue beyond the expiration date without committee approval. Failure to receive approval for continuation before the expiration date will result in the automatic suspension of the approval of this protocol on the expiration date. Information collected following suspension is unapproved research and cannot be reported or published as research data. If you do not wish continued approval, please notify the Committee of the study termination.

This approval by the Biosci IRB does not replace or supersede any departmental or oversight committee review that may be required by institutional policy.

Adverse Reactions: If any untoward incidents or severe reactions should develop as a result of this study, you are required to notify the Biosci IRB immediately. If necessary a member of the IRB will be assigned to look into the matter. If the problem is serious, approval may be withdrawn pending IRB review.

Amendments: If you wish to change any aspect of this study, such as the procedures, the consent forms, or the investigators, please communicate your requested changes to the Biosci IRB. The new procedure is not to be initiated until the IRB approval has been given.

Please retain a copy of this letter with your approved protocol.

APPENDIX D

RECRUITMENT ADVERTISEMENT

Can your iPHONE help you eat better and lose weight?
HELP US FIND OUT
INVESTIGATORS FROM THE NUTRITION PROGRAM AT ARIZONA STATE UNIVERSITY
ARE RECRUITING VOLUNTEERS (20-70 y of age) to test iPhone diet apps

IF YOU CURRENTLY OWN AN iPHONE AND ARE OVERWEIGHT AND DESIRE TO LOSE WEIGHT, YOU MAY QUALIFY FOR THIS TRIAL

THIS STUDY WILL EVALUATE THE USEFULNESS OF iPHONES APPS AS TOOLS TO HELP INDIVIDUALS ADHERE TO WEIGHT LOSS DIETS

Participation will include:

- Using the iPhone app daily for 8 weeks to record food consumed
- Meeting with trial investigators on four occasions at the ASU Downtown campus
- Being weighed and measured, and providing fasting blood samples on 3 occasions

You will receive the app at no charge during the trial and $30 in gift cards to Target if you participate in this trial
INTERESTED? Please complete our online survey at: https://www.surveymonkey.com/s/ASUiPhoneStudy

APPENDIX E

HEALTH HISTORY QUESTIONNAIRE

HEALTH /HISTORY QUESTIONNAIRE ID#_____

1. Gender: M F Age: _____ To be completed → by study personnel Weight _____ Height _____ Waist _____

2. Have you lost or gained *more than* 5 lbs in the last 3 months? Yes No

   If yes, how many pounds lost or gained? _____ How long ago? _____

   Do you desire to lose weight? Yes No How many pounds? _____

3. Do you follow a special diet? (weight gain/loss, vegetarian, low-fat, etc.) Yes No

   If yes, please explain:

   _____

4. Are you willing to record food consumed on a daily basis for 8 weeks on your iPhone? Yes No

5. Education (please circle) High school Some college College graduate

6. Ethnicity: (please circle one) Hispanic or Latino Not Hispanic or Latino

7. Race: (please circle) American Indian/Alaska Native African-American White
   Native Hawaiian/Other Pacific Islander Asian Other

8. Do you smoke? No, never _____
   Yes _____ # Cigarettes per day = _____
   I used to, but I quit _____ months/years (circle) ago

9. Do you take any medications regularly? Yes No

If yes, list type and frequency:

Medication	Dosage	Frequency

10. Do you currently take supplements (vitamins, minerals, herbs, etc.) ? Yes No
If yes, list type and frequency:

Supplement	Dosage	Frequency

11. Are you OK with providing a fasting blood sample (~2 TBLS) on 3 occasions during the study? Yes No

12. How much alcohol do you drink? (average drinks per day) _____

13. Please ANSWER (YES) if **you have ever** been diagnosed with any of the following diseases or symptoms:

	YES		YES
Coronary Heart Disease		Chest Pain	
High Blood Pressure		Shortness of Breath	
Heart Murmur		Heart Palpitations	
Rheumatic Fever		Any Heart Problems	
Irregular Heart Beat		Coughing of Blood	
Varicose Veins		Feeling Faint or Dizzy	
Stroke		Lung Disease	
Diabetes		Liver Disease	
Low Blood Sugar		Kidney Disease	
Bronchial Asthma		Thyroid Disease	
Hay Fever		Anemia	
Leg or Ankle Swelling		Hormone Imbalances	
Eating Disorders		Emotional Problems	

Please elaborate on any condition listed above

_____

_____

_____

14. How would you rate your lifestyle? (please check)

Not active _____ Active _____

Somewhat active _____ Very Active _____

15. Please circle **the number of times** you did the following kinds of exercises **for more than 15 minutes** last week.

Mild exercise (minimal effort):
Easy walking, golf, gardening, bowling, yoga, fishing, horseshoes, archery, etc.

Times per week: 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14+

Moderate exercise (not exhausting):
Fast walking, easy bicycling, tennis, easy swimming, badminton, dancing, volleyball, baseball, etc.

Times per week: 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14+

Strenuous exercise activities (heart beats rapidly):
Running, jogging, hocky, football, soccer, squash, basketball, cross country skiing, judo, roller skating, vigorous swimming, vigorous long distance bicycling, etc.

Times per week: 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14+

16. Do you have any food allergies? Yes No *If yes, please explain*:

_____

APPENDIX F

PHYSICAL ACTIVITY QUESTIONNAIRE

Godin Leisure-Time Exercise Questionnaire

1. During a typical **7-Day period** (a week), how many times on the average do you do the following kinds of exercise for **more than 15 minutes** during your free time (write on each line the appropriate number).

	Times Per Week:
a) STRENUOUS EXERCISE **(HEART BEATS RAPIDLY)** (e.g., running, jogging, hockey, football, soccer, squash, basketball, cross country skiing, judo, roller skating, vigorous swimming, vigorous long distance bicycling)	_____
b) MODERATE EXERCISE **(NOT EXHAUSTING)** (e.g., fast walking, baseball, tennis, easy bicycling, volleyball, badminton, easy swimming, alpine skiing, popular and folk dancing)	_____
c) MILD EXERCISE **(MINIMAL EFFORT)** (e.g., yoga, archery, fishing from river bank, bowling, horseshoes, golf, snow-mobiling, easy walking)	_____

2. During a typical **7-Day period** (a week), in your leisure time, how often do you engage in any regular activity **long enough to work up a sweat** (heart beats rapidly)?

OFTEN	SOMETIMES	NEVER/RARELY
1. ☐	2. ☐	3. ☐

APPENDIX G

EXIT SURVEY

ID#_____ ____

EXIT SURVEY: Please answer the following questions regarding your participation in the research study on successful weight loss strategies.

Mark an 'X' on the line best fitting your opinion.

1. Using the iPhone app was helpful in keeping me on track toward my weight loss goal.

strongly disagree | disagree | no opinion | agree | strongly agree

2. The app I used for recording my daily food intake was too time consuming to be practical.

strongly disagree | disagree | no opinion | agree | strongly agree

3. I was more aware of my eating habits because I was recording my food intake.

strongly disagree | disagree | no opinion | agree | strongly agree

4. I am more confident in my ability to lose weight after participating in this study.

strongly disagree | disagree | no opinion | agree | strongly agree

5. I have learned much about my food habits and my eating has improved by participating in this study.

strongly disagree | disagree | no opinion | agree | strongly agree

6. I will continue to record my food intake after the study is over.

strongly disagree | disagree | no opinion | agree | strongly agree

7. Having a dietitian providing feedback to my diet on a daily basis was (or would have been) beneficial.

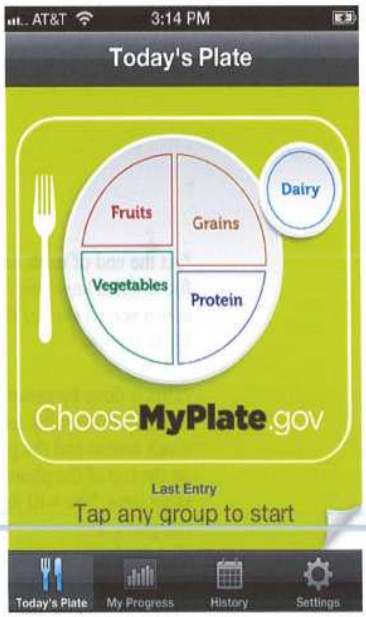
strongly disagree | disagree | no opinion | agree | strongly agree

Comments regarding study participation/expectations:

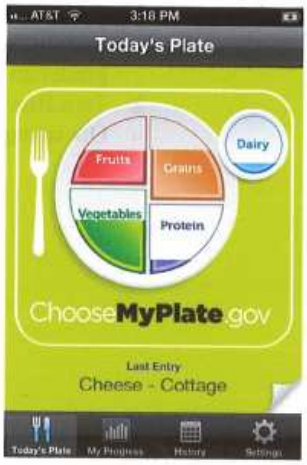
APPENDIX H

APP INSTRUCTIONS MP-C

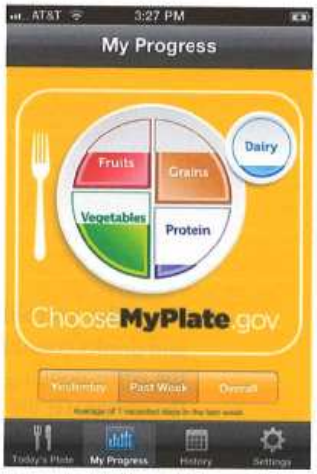
1. Go to the settings tab and enter in the appropriate gender and year born.
2. Turn Bravo Alerts to the ON setting.



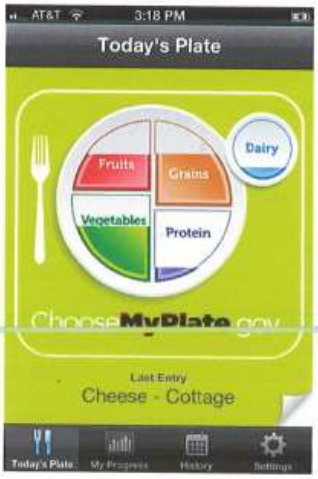
-Each day you will record all food and drinks consumed, by pressing the corresponding food group on the screen. (If the food item is not available in the database select a similar item that is available in the database.)



-The corresponding food group section will start to fill up as you add new items.
-The goal is to fill all sections of the plate.



-The My Progress tab allows you to see previous days and weeks along with an overall picture of your daily diet.



*At the end of **each day**, when all foods eaten have been entered take a screen shot of the **Today's Plate** section.*

-This is done by pressing the round button on the front of the touch screen and the power button on the top of the phone at the same time. You will then email this photo to:
claudia.thompson@asu.edu

This will be done everyday for the duration of the trial.

APPENDIX I

APP INSTRUCTIONS PIC-C

This is is the home screen. From the home screen you can access all of the features of the app.

Daily Parameters: Everyday you will enter your weight, hours of sleep, sleep quality, energy level, and stress level by tapping that button.

Meal Input: Everyday all meals and drinks will be entered into the app by selecting meal input. This will allow you to take a picture of all of the food items you consume.

Today's Timeline: This section will show a running list of all of the food items you have entered throughout the day so you may review them at anytime throughout the day.

Physical Activity: If you complete physical activity, log it by tapping that button.

Reports: This is where you will receive daily feedback from a registered dietitian. The R.D. will be evaluating your diet from the information you have provided. Each day you will receive feedback on the previous days diet. This button will also allow you to look at all previous data entered into the MyDietitian app.

Video Library: This section provides a wide variety of short educational videos. The videos were created by nutrition professionals. Examples are: FAD Diets, Fiber, Alcohol,Metabolism, Office Snacking....If you feel like you need help in a specific area, there is a good chance a video on that topic has been provided.

1. Once the app has been loaded onto your phone you will click the sign up button to create an account. Use an email account that you check regularly.

2. Next, you will click the settings tab at the bottom of the app. You will want to click on the User Account tab to enter your personal information.

3. When in the User Account tab you will enter your name, date of birth, gender, current weight, and your personal goal for using this phone application. Once the data has been entered hit save.

APPENDIX J

APP INSTRUCTIONS PIC-RD

Press the tour button at the bottom of the home screen, and watch the instructional video on the use of the MyDietitian phone application.

1. Once the app has been loaded onto your phone you will click the sign up button to create an account. Use an email account that you check regularly.

2. Next, you will click the settings tab at the bottom of the app. You will want to click on the User Account tab to enter your personal information.

3. When in the User Account tab you will enter your name, date of birth, gender, current weight, and your personal goal for using this phone application. Once the data has been entered hit save.

This is is the home screen. From the home screen you can access all of the features of the app.

Daily Parameters: Everyday you will enter your weight, hours of sleep, sleep quality, energy level, and stress level by tapping that button.

Meal Input: Everyday all meals and drinks will be entered into the app by selecting meal input. This will allow you to take a picture of all of the food items you consume.

Today's Timeline: This section will show a running list of all of the food items you have entered throughout the day so you may review them at anytime throughout the day.

Physical Activity: If you complete physical activity, log it by tapping that button.

Reports: This is where you will receive daily feedback from a registered dietitian. The R.D. will be evaluating your diet from the information you have provided. Each day you will receive feedback on the previous days diet. This button will also allow you to look at all previous data entered into the MyDietitian app.

Video Library: This section provides a wide variety of short educational videos. The videos were created by nutrition professionals. Examples are: FAD Diets, Fiber, Alcohol,Metabolism, Office Snacking....If you feel like you need help in a specific area, there is a good chance a video on that topic has been provided.

APPENDIX K

HEMOGLOBIN A1C

SIEMENS

DCA™ Systems

Hemoglobin A_{1c} Reagent Kit

For Use With DCA™ Analyzers

A Quantitative Assay for Hemoglobin A_{1c} in Blood

83449439 Rev. A, 2011-03

INTENDED USE:
This assay provides a convenient, quantitative method for measuring the percent concentration of hemoglobin A_{1c} in blood. The measurement of hemoglobin A_{1c} concentration is recommended for monitoring the long-term care of persons with diabetes.

The Diabetes Control and Complications Trial (DCCT) showed the importance of improved glycemic control in reducing the risk and progression of the complications of diabetes.[1] Glycemic control was determined by the measurement of hemoglobin A_{1c}. The American Diabetes Association (ADA) recommends measurement of hemoglobin A_{1c} levels two to four times per year, less frequently in patients with stable control.[2]

This assay is based on a latex immunoagglutination inhibition methodology.[3] After loading the reagent test cartridge into the DCA™ Analyzer, the test result is displayed in six minutes.

The DCA Hemoglobin A_{1c} assay is for use in laboratories such as physician office laboratories, clinics, and hospitals.

INFORMATION REGARDING CLIA WAIVER (US ONLY):
The DCA Vantage system is CLIA-waived only when used with Siemens-branded DCA 2000+ or DCA Systems HbA_{1c} cartridges.

A certificate of CLIA waiver is required to perform the test in a waived setting.

To obtain a Certificate of Waiver, contact your state department of health or visit the CMS website for an application, form CMS-116.

Failure to adhere to the instructions for use, including instructions for limitations or intended use, and for performing QC testing, is considered as off-label use, resulting in the test being categorized as high complexity and subject to all CLIA regulation.[4]

SUMMARY AND EXPLANATION:
Hemoglobin A_{1c} is formed by the non-enzymatic glycation of the N-terminus of the ß-chain of hemoglobin Ao.[4] The level of hemoglobin A_{1c} is proportional to the level of glucose in the blood over a period of approximately two months.[4] Thus, hemoglobin A_{1c} is accepted as an indicator of the mean daily blood glucose concentration over the preceding two months.[5,7] Studies have shown that the clinical values obtained through regular measurement of hemoglobin A_{1c} lead to changes in diabetes treatment and improvement of metabolic control as indicated by a lowering of hemoglobin A_{1c} values.[4,5]

CHEMICAL PRINCIPLES OF PROCEDURE:
Both the concentration of hemoglobin A_{1c} specifically and the concentration of total hemoglobin are measured, and the ratio reported as percent hemoglobin A_{1c}.[6]

All of the reagents for performing both reactions are contained in the DCA Hemoglobin A_{1c} (HbA_{1c}) REAGENT CARTRIDGE Reagent Cartridge (Figure 1).

DCA HbA_{1c} Reagent Cartridge

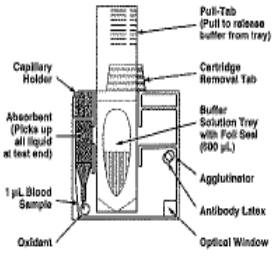
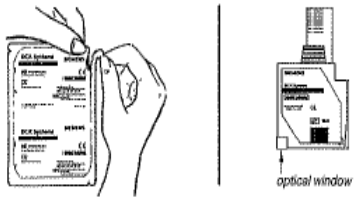


Figure 1

RECOMMENDED PROCEDURES FOR HANDLING REAGENT CARTRIDGES:
To open the foil pouch, tear down from the corner notch (until the entire long side of the pouch is open).



Discard the reagent cartridge if the cartridge is damaged, the pull-tab is loose or missing, the desiccant is missing, or if loose desiccant particles are found inside the foil pouch.

Upon removal from refrigerated storage, allow the reagent cartridge to warm up at room temperature for 10 minutes (in the unopened foil pouch) or 5 minutes (if removed from the foil pouch). **After opening the foil pouch, the reagent cartridge must be used within (1) hour.**

RECOMMENDED PROCEDURES FOR HANDLING CAPILLARY HOLDERS:
Unused capillary holders may be saved and used with any lot of reagent cartridges. Each capillary holder is packaged separately in a blister package. To remove the capillary holder, remove the white plastic film from the clear plastic blister. DO NOT PUSH ⃠ the capillary holder out of or through the plastic.

Discard the plastic capillary holder if any of the following are missing from the holder: (a) glass capillary, (b) absorbent pad, (c) latching mechanism.

STABILITY OF REAGENT CARTRIDGES:
Do not use reagent cartridges after the last day of the expiration month.

SPECIMEN COLLECTION AND PREPARATION:
The provided glass capillary (within plastic capillary holder) holds 1 µL of whole blood. The blood sample may be obtained by finger stick or venipuncture. Acceptable anticoagulants are EDTA, heparin, flouride/oxalate, and citrate.
Important: After the glass capillary is filled with sample, analysis must begin within 5 minutes.

EDTA, heparin, flouride/oxalate, and citrate preserved whole blood may be stored at -70° to 5°C (-94° to 41°F) for two weeks, or up to 25°C (77°F) for one week.

Do not refreeze previously frozen blood samples or store in a self-defrosting freezer. Allow blood sample to reach room temperature. Mix blood sample thoroughly before use.

TESTING PROCEDURE:
See the Quick Reference Guide and Operator's Guide for detailed illustrated directions.

CALIBRATION:
Instrument: The DCA Analyzer is calibrated by the manufacturer. Thereafter, the instrument automatically self-adjusts during first-time power-up and during

All laboratory tests are subject to random error. If the test result is questionable, or if clinical signs and symptoms appear inconsistent with test results, re-assay the sample or confirm the result using another method.

LIMITATIONS OF PROCEDURE:

The DCA HbA_{1c} assay gives accurate and precise results over a range of total hemoglobin of 7 to 24 g/dL. Most patients will have hemoglobin concentrations within these values. However, patients with severe anemias may have hemoglobin concentrations lower than 7 g/dL, and patients with polycythemia may have hemoglobin concentrations above 24 g/dL. Patients known to have these conditions should be assayed by a test employing a different assay principle if their hemoglobin concentrations are outside of the acceptable range.

Glycated hemoglobin F is not measured by the DCA HbA_{1c} assay. At levels of hemoglobin F less than 10%, the DCA system accurately indicates the patient's glycemic control. However, at very high levels of hemoglobin F (> 10%), the amount of HbA_{1c} is lower than expected because a greater proportion of the glycated hemoglobin is in the form of glycated hemoglobin F. HbA_{1c} results for such patients do not accurately indicate the patient's glycemic control and should not be compared to published normal or abnormal values.

Conditions such as hemolytic anemia, polycythemia, homozygous HbS, and HbC, can result in decreased life span of the red blood cells, which causes HbA_{1c} results to be lower than expected, regardless of the method used, and not be related to glycemic control, when using published reference ranges.

Bilirubin, up to a level of 20 mg/dL, does not interfere with this assay.

Triglycerides, up to 1347 mg/dL in fresh whole blood, do not interfere with this assay. Highly lipemic blood samples stored for long periods of time or frozen should not be assayed using this method.

Rheumatoid factor, up to 1:5120 titer, does not interfere with this assay.

Expected serum levels of the following drugs commonly prescribed to persons with diabetes do not interfere with this assay: Diabinese, Orinase, Tolinase, Micronase, Dymelor, glipizide.

EXPECTED VALUES:

The expected normal range for % HbA_{1c} using the DCA HbA_{1c} test was determined by assaying blood samples from 103 apparently healthy individuals (fasting blood glucose < 120 mg/dL). No significant differences in normal range were observed among males and females, geographical location, or age groups evaluated. The mean HbA_{1c} value was 5.0% ± 0.35% (1 S.D.). The range was 4.2% to 6.5%. The 95% confidence limits (mean ± 2 S.D.) were 4.3% to 5.7%. These values are similar to those reported in the literature.[7]

Depending on the assay methodology used, HbA_{1c} is approximately 3% to 6% in non-diabetics, 6% to 8% in controlled diabetics and can be as much as 20% or higher in poorly controlled diabetics.[8] However, each laboratory should determine normal ranges to conform with the population being tested.

SPECIFIC PERFORMANCE CHARACTERISTICS:

The precision and correlation data are results of studies conducted by the staff at separate physician offices. The statistical calculations were performed following Clinical Laboratory Standards Institute (CLSI) procedures.

Precision: Multiple DCA 2000 HbA_{1c} assays of two different commercially prepared whole blood controls were performed by three independent investigators. The assigned values listed were determined from studies conducted by the manufacturer. Within-run precision was evaluated by including Normal and Abnormal controls, in duplicate, in each run of clinical specimens.

Control	Site No.	Assigned Value (HbA_{1c})	Mean Value (HbA_{1c})	No. Runs	No. Assays	Within-Run S.D.	Within-Run %C.V.	Between-Run S.D.	Between-Run %C.V.
Normal	1	5.2	4.95	21	42	0.16	3.3	Neg.*	Neg.*
Normal	2	5.2	5.10	22	44	0.11	2.2	0.06	1.2
Normal	3	5.2	5.11	22	44	0.12	2.3	0.06	1.1
Abnormal	1	11.9	11.32	21	42	0.34	3.0	Neg.*	Neg.*
Abnormal	2	11.9	11.86	22	44	0.33	2.8	0.51	4.3
Abnormal	3	11.9	11.81	22	44	0.44	3.7	0.11	0.9

*Negligible

Correlation: The percentage of HbA_{1c} in clinical specimens ranging from 3.8% to 14.0% HbA_{1c} (both venous and capillary) was determined using the DCA 2000 HbA_{1c} System (y) and ion exchange high performance liquid chromatography (HPLC) (x). Results are as follows:

Site No.	Sample Type	No. of Assays	Regression Line	Standard Error of Estimate	Correlation Coefficient
1	venous	50	$y = 0.91x + 0.26$	0.42	0.98
1	capillary	50	$y = 0.94x + 0.00$	0.51	0.98
2	venous	47	$y = 0.89x + 0.42$	0.39	0.98
2	capillary	47	$y = 0.91x + 0.34$	0.50	0.97
3	venous	49	$y = 0.94x + 0.34$	0.42	0.98
3	capillary	50	$y = 0.91x + 0.58$	0.52	0.97

In addition, a correlation study was performed at a university diabetes center using the DCA 2000 HbA_{1c} System (y) and a reference HPLC[9] (x) used during the DCCT:

Site No.	Sample Type	No. of Assays	Regression Line	Standard Error of Estimate	Correlation Coefficient
4	venous	100	$y = 1.02x - 0.00$	0.45	0.98

CLIA WAIVER ACCURACY:

To evaluate the expected performance of the Siemens Healthcare Diagnostics DCA Hemoglobin A_{1c} product used on the DCA 2000 analyzer in a CLIA-waived setting, a lay user field study was performed at three non-laboratory study sites. The 68 participants represented diverse demographics, had no previous laboratory experience, and received no training for the study. Participants were provided with six (6) masked whole blood hemolysates with established target concentrations for HbA_{1c} to be used as patient specimens: 4.36, 6.25, 8.18, 8.88, 9.94, and 11.63% HbA_{1c}. The lay user results were compared to target values traceable to the high pressure liquid chromatography (HPLC) reference method used at the Glycohemoglobin Reference Laboratory at the University of Missouri Medical Center.

A summary of the performance is shown below.
Lay User Results: 408 Lay Users: 68
The overall accuracy and imprecision rates for HbA_{1c} were:

Target Level (% HbA_{1c})	Accuracy Mean (% HbA_{1c})	Accuracy 95% CI*	Imprecision SD	Imprecision % CV
4.36	4.35	4.30–4.40	0.24	5.4
6.25	6.14	6.10–6.18	0.18	2.9
8.18	8.19	8.04–8.34	0.33	4.0
8.88	8.97	8.90–9.04	0.28	3.2
9.94	9.96	9.88–10.02	0.30	3.0
11.63	11.71	11.61–11.81	0.39	3.4

*95% Confidence Interval
Statistical analysis (t-statistics) demonstrated that the observed differences among the three study sites were not significant.

Figure 1

For the measurement of total hemoglobin, potassium ferricyanide is used to oxidize hemoglobin in the sample to methemoglobin. The methemoglobin then complexes with thiocyanate to form thiocyan-methemoglobin, the colored species that is measured. The extent of color development at 531 nm is proportional to the concentration of total hemoglobin in the sample.

For the measurement of specific HbA_{1c}, an inhibition of latex agglutination assay is used (Figure 2).

Assay Principle
Inhibition Of Latex Agglutination

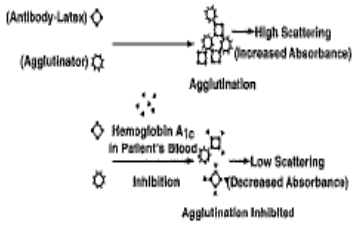


Figure 2

An agglutinator (synthetic polymer containing multiple copies of the immunoreactive portion of HbA_{1c}) causes agglutination of latex coated with HbA_{1c} specific mouse monoclonal antibody. This agglutination reaction causes increased scattering of light, which is measured as an increase in absorbance at 531 nm. HbA_{1c} in whole blood specimens competes for the limited number of antibody-latex binding sites causing an inhibition of agglutination and a decreased scattering of light. The decreased scattering is measured as a decrease in absorbance at 531 nm. The HbA_{1c} concentration is then quantified using a calibration curve of absorbance versus HbA_{1c} concentration.

The *percent* HbA_{1c} in the sample is then calculated as follows:

$$\%\ HbA_{1c} = \frac{[HbA_{1c}]}{[\text{Total Hemoglobin}]} \times 100$$

The IFCC concentration in mmol/mol HbA_{1c} is calculated as follows:

HbA_{1c} mmol/mol = (HbA_{1c} mmol)/(Total hemoglobin mol)

All measurements and calculations are performed automatically by the DCA Analyzer, and the screen displays the HbA_{1c} at the end of the assay. Values in this insert are in % HbA_{1c} NGSP and where shown in parentheses, as mmol/mol HbA_{1c} IFCC.

KIT CONTENTS:
- 10 Reagent Cartridges
- 11 Capillary Holders
- Calibration Card
- 2 Package Inserts

REAGENTS:

Antibody Latex: HbA_{1c}-specific mouse monoclonal antibody adsorbed onto latex particles. 2.5% w/v antibody-latex in 10 mM glycine buffer; 16% w/v nonreactive ingredients (10 µL dried in each reagent cartridge).

Agglutinator: 0.005% w/v poly (aspartic acid) polymer covalently attached to the HbA_{1c} hapten in 20 mM sodium citrate buffer containing 0.1% w/v bovine serum albumin and 1% w/v nonreactive ingredients (10 µL dried in each cartridge).

Buffer Solution: 8.1% w/v lithium thiocyanate, 0.01% digitonin in 200 mM glycine buffer (0.6 mL in each cartridge).

Oxidant: 1.5% w/v potassium ferricyanide in water with 21% w/v nonreactive ingredients (10 µL dried in each cartridge).

CAUTION:
- DCA HbA_{1c} Reagent Cartridges are for IVD *in vitro* diagnostic use.
- Safety glasses, gloves and lab coat are recommended when using the DCA System.

WARNING:
- To prevent injury, do not force removal of a cartridge from the instrument. Consult the operator's guide to verify the proper removal technique. Contact your technical service provider if the problem cannot be solved.

TEMPERATURE INDICATOR:

Upon receipt of this kit, check the temperature indicator located on the front of the carton. If the indicator has turned red, do not use the reagent cartridges. Note time and date received, and for assistance in obtaining a replacement kit, refer to instructions given on the carton.

STORAGE:

2°C-8°C Store reagent cartridges refrigerated at 2° to 8°C (36° to 46°F).

15°C-30°C Capillary holders may be stored refrigerated or at room temperature (15° to 30°C/59° to 86°F).

USE LIFE:

Reagent cartridges can be kept for up to three months at room temperature anytime before the (EXP) expiration date. Record on the carton, the date the carton was placed at room temperature.

the instrument automatically self-adjusts during first-time power-up and during each assay. In the event the system is unable to make appropriate internal adjustments, an error message is displayed.

Reagent: Before reagent cartridges are released by the manufacturer, each lot of reagent cartridges undergoes a thorough analysis and characterization. Values of calibration parameters based on a DCCT reference method are determined that provide for optimal reagent performance. DCA HbA_{1c} test method is National Glycohemoglobin Standardization Program (NGSP) Certified and is traceable to International Federation of Clinical Chemistry (IFCC) reference materials and test methods. The values for the calibration parameters are encoded onto the calibration card provided with each lot of reagent cartridges. Prior to use of each new lot of reagent cartridges, scan the calibration card into the analyzer.

Before the sample can be analyzed, the reagent cartridge barcode (containing lot number and test name) is scanned. This accesses the appropriate calibration parameter values (calibration curve) for the particular lot number of reagent cartridges in use. If no calibration curve is in the instrument for the particular lot number of cartridges in use, the instrument prompts the user to scan the calibration card.

The instrument can store two calibrations for the DCA HbA_{1c} Assay. Each of two calibrations is for a different lot number.

When reagent cartridges are stored and used properly, acceptable performance up to the expiration date is ensured. To verify proper functioning of the DCA System, analyze DCA HbA_{1c} Controls (refer to Quality Control section).

QUALITY CONTROL:

To assure quality of both testing procedures and patient results for hemoglobin A_{1c}, the DCA System performs 48 optical, electronic, mechanical, and reagent systems checks during the course of each specimen assay. These checks include calibration verification during every test. If an assay or system error occurs during any individual measurement, the system automatically reports an error message, preventing the reporting of erroneous patient results.

CLIA WAIVED LABORATORIES:

It is recommended that quality control specimens be tested with each new lot of reagents, new shipment of reagents and monthly for reagents that have been stored for more than 30 days. QC testing is recommended to ensure reagent storage integrity, train and confirm performance acceptability for new users, and when patients' clinical conditions or symptoms do not match. Additional QC intervals may be required as per your laboratory procedures. Liquid ready-to-use controls are available; contact technical support for recommendations.

Compare QC results to those listed as acceptable by the QC manufacturer. If control results are not acceptable, do not test patient samples until the problem is resolved. Repeat control testing until results are acceptable.

For technical support assistance call (877) 229-3711.

ALL OTHER LABORATORIES:

The staff at each laboratory site can benefit by establishing a quality assurance plan, based on their institution's policies. Run quality control specimens under the following conditions:
- At regular intervals determined by the laboratory procedures
- With each new shipment of reagents
- With each new lot of reagents
- Each time a calibration card is scanned
- To train and confirm performance acceptability for new analysts
- When results do not match the patient's clinical condition or symptoms.

Good laboratory practices include a well-designed and implemented quality control process. These practices, for example, may involve:
- Proper storage and handling of reagent kits
- Careful sample collection and handling procedures
- Training of testing personnel
- Routine review of sample and control results
- Periodic quality system reviews
- Retention of quality control testing records.

If the problem cannot be corrected, or the reason for an out-of-limits result cannot be determined, contact the Authorized Representative nearest you.

RESULTS:

The displayed test result requires no further calculation. HbA_{1c} concentrations in the following range are reported: 2.5% to 14.0% HbA_{1c} NGSP (HbA_{1c} range 4 mmol/mol to 130 mmol/mol IFCC).

The test is linear throughout this range.

Result preceded by a less than sign (<):

A less than sign in the display indicates a concentration below the lower limit of the test (under range). Report the result as being less than 2.5% HbA_{1c} NGSP (4 mmol/mol HbA_{1c} IFCC). This method does not provide for re-assay using a larger sample aliquot. Results less than 2.5% HbA_{1c} NGSP (4 mmol/mol HbA_{1c} IFCC) are rare and may indicate that the sample contains substantial amounts of fetal hemoglobin (does not react in the immunoassay); or that the patient may be suffering from hemolytic anemia or polycythemia (conditions which often result in a significant decrease in the life span of red blood cells).

Result preceded by a greater than sign (>):

A greater than sign in the display indicates a concentration above the upper limit of the test (over range). Report the result as being more than 14.0% HbA_{1c} NGSP (130 mmol/mol HbA_{1c} IFCC). This method does not provide for re-assay using a diluted sample. To obtain a more quantitative test value, use another test method.

Specificity:

Effect of Hemoglobin Variants: The antibody in the DCA HbA_{1c} assay is specific for the first few amino acid residues of the glycated amino-terminus of the ß-chain of hemoglobin A. Any glycated hemoglobin molecule having this same structure will be measured in the assay. Most glycated hemoglobin variants are immunoreactive in the DCA HbA_{1c} assay (such as, HbS_{1c}, HbC_{1c}, HbE_{1c}). The point mutations in these molecules occur at the 6 position of the ß-chain (HbS and HbC) and at the 26 position of the ß-chain (HbE). Thus, the point mutations in these variants do not affect the binding of the antibody used in the DCA HbA_{1c} assay. The DCA reports %HbA_{1c} values that reflect the glycemic control of patients with these hemoglobinopathies.[13,14]

Effect of Pre-HbA_{1c} (Labile Fraction): The labile fraction (Schiff base attachment of glucose to HbA, or pre-HbA_{1c}) does not affect the assay result because the antibody is specific for the stable ketoamine.[13]

Effect of Carbamylated Hemoglobin: Carbamylated hemoglobin (elevated in patients with uremia) does not affect the assay result because the antibody is specific for the sugar moiety of HbA_{1c}.[15,16]

AVAILABILITY:

DCA HbA_{1c} Reagent Kit is available as **REF** 5035C (10's). DCA CONTROL NORMAL Normal and CONTROL ABNORMAL Abnormal Control kit is available as **REF** 5068A.

GLOSSARY OF ACRONYMS

ADA: American Diabetes Association • **CLIA:** Clinical Laboratory Improvement Amendments • **CLSI:** Clinical and Laboratory Standards Institute
DCCT: Diabetes Control and Complications Trial • **IFCC:** International Federation of Clinical Chemistry • **NGSP:** National Glycohemoglobin Standardization Program

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(株)

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CE

APPENDIX L

CHOLESTEROL

04773349001V4

CHOL2

Cholesterol Gen.2

cobas®

• Indicates **cobas c** systems on which reagents can be used

Order information			**cobas c** systems
			cobas c 111
Cholesterol Gen.2			
4 x 100 tests	Cat. No. **04718917** 190	CHO2I: ACN 798 CHO2A: ACN 433	•
Calibrator f.a.s. (12 x 3 mL)	Cat. No. **10759350** 190	Code 401	
Calibrator f.a.s. (12 x 3 mL, for USA)	Cat. No. **10759350** 360	Code 401	
Precinorm U plus (10 x 3 mL)	Cat. No. **12149435** 122	Code 300	
Precinorm U plus (10 x 3 mL, for USA)	Cat. No. **12149435** 160	Code 300	
Precipath U plus (10 x 3 mL)	Cat. No. **12149443** 122	Code 301	
Precipath U plus (10 x 3 mL, for USA)	Cat. No. **12149443** 160	Code 301	
Precinorm U (20 x 5 mL)	Cat. No. **10171743** 122	Code 300	
Precipath U (20 x 5 mL)	Cat. No. **10171778** 122	Code 301	
Precinorm L (4 x 3 mL)	Cat. No. **10781827** 122	Code 304	
Precipath L (4 x 3 mL)	Cat. No. **11285874** 122	Code 305	

English

Intended use

In vitro test for the quantitative determination of cholesterol in human serum and plasma on the **cobas c 111** system.

Summary

Cholesterol is a steroid with a secondary hydroxyl group in the C3 position. It is synthesized in many types of tissue, but particularly in the liver and intestinal wall. Approximately three quarters of cholesterol is newly synthesized and a quarter originates from dietary intake. Cholesterol assays are used for screening for atherosclerotic risk and in the diagnosis and treatment of disorders involving elevated cholesterol levels as well as lipid and lipoprotein metabolic disorders.

Cholesterol analysis was first reported by Liebermann in 1885 followed by Burchard in 1889. In the Liebermann-Burchard reaction, cholesterol forms a blue-green dye from polymeric unsaturated carbohydrates in an acetic acid/acetic anhydride/concentrated sulfuric acid medium. The Abell and Kendall method is specific for cholesterol, but is technically complex and requires the use of corrosive reagents. In 1974, Roeschlau and Allain described the first fully enzymatic method. This method is based on the determination of Δ4-cholestenone after enzymatic cleavage of the cholesterol ester by cholesterol esterase, conversion of cholesterol by cholesterol oxidase, and subsequent measurement by the Trinder reaction of the hydrogen peroxide formed. Optimization of ester cleavage (> 99.5 %) allows standardization using primary and secondary standards and a direct comparison with the CDC and NIST reference methods.[1,2,3,4,5,6,7,8,9] Nonfasting sample results may be slightly lower than fasting results.[10,11]

The Roche cholesterol assay meets the 1992 National Institutes of Health (NIH) goal of less than or equal to 3 % for both precision and bias.[12]

The assay is optionally standardized against Abell/Kendall and isotope dilution/mass spectrometry. The performance claims and data presented here are independent from the standardization.

Test principle

Enzymatic, colorimetric method.

Cholesterol esters are cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids. Cholesterol oxidase then catalyzes the oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide formed effects the oxidative coupling of phenol and 4-aminoantipyrine to form a red quinone-imine dye.

$$\text{Cholesterol esters} + H_2O \xrightarrow{CE} \text{cholesterol} + \text{RCOOH}$$

$$\text{Cholesterol} + O_2 \xrightarrow{CHOD} \text{cholest-4-en-3-one} + H_2O_2$$

$$2\,H_2O_2 + \text{4-AAP} + \text{phenol} \xrightarrow{POD} \text{quinone-imine dye} + 4\,H_2O$$

The color intensity of the dye formed is directly proportional to the cholesterol concentration. It is determined by measuring the increase in absorbance.

Reagents - working solutions

R1 PIPES buffer: 225 mmol/L, pH 6.8; Mg^{2+}: 10 mmol/L; sodium cholate: 0.6 mmol/L; 4-aminoantipyrine: ≥ 0.45 mmol/L; phenol: ≥ 12.6 mmol/L; fatty alcohol polyglycol ether: 3 %; CE (Pseudomonas spec.): ≥ 25 µkat/L (≥ 1.5 U/mL); CHOD (E. coli): ≥ 7.5 µkat/L (≥ 0.45 U/mL); POD (horseradish): ≥ 12.5 µkat/L (≥ 0.75 U/mL); stabilizers; preservative

Precautions and warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.

Safety data sheet available for professional user on request.

Disposal of all waste material should be in accordance with local guidelines.

Reagent handling

Ready for use.

Inaccurate pipetting of reagent, leading to potentially erroneous results, may be caused by excessive foaming of this reagent. Ensure that foam is removed from the surface of the reagent prior to setting the reagent in the analyzer.

Storage and stability

CHOL2

Shelf life at 2-8 °C:	See expiration date on reagent
On-board in use and refrigerated on the analyzer:	4 weeks

Specimen collection and preparation

For specimen collection and preparation, only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum.

Plasma: Li-heparin, K_3-EDTA plasma.

(Use of EDTA plasma leads to slightly lower values).

Do not use citrate, oxalate, or fluoride.[13]

Fasting and nonfasting samples can be used.[11]

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability:[14,15] 7 days at 15-25 °C
7 days at 2-8 °C
3 months at (-15)-(-25) °C

Materials provided

See "Reagents - working solutions" section for reagents.

Materials required (but not provided)

See "Order information" section.

CHOL2

Cholesterol Gen.2

cobas®

Deionized water
General laboratory equipment

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

cobas c 111 test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction direction	Increase
Wavelength A/B	512/659 nm
Calc. first/last	6/37
Unit	mmol/L
Reaction mode	R-S

Pipetting parameters

		Diluent (H_2O)
R	47 µL	70 µL
Sample	2 µL	23 µL
Total volume	142 µL	

Calibration

Calibrator	Calibrator f.a.s. Deionized water is used automatically by the instrument as the zero calibrator.
Calibration mode	Linear regression
Calibration interval	Each lot and as required following quality control procedures

Traceability: This method has been standardized according to Abell/Kendall[12] and also by isotope dilution/mass spectrometry[15].
This complies with the requirements of the National Institute of Standards and Technology (NIST).

Quality control

For quality control, use control materials as listed in the "Order information" section.
Other suitable control material can be used in addition.
The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits.
Each laboratory should establish corrective measures to be taken if values fall outside the limits.
Follow the applicable government regulations and local guidelines for quality control.

Calculation

The **cobas c** 111 analyzer automatically calculates the analyte concentration of each sample.

Conversion factors: mmol/L x 38.66 = mg/dL
mmol/L x 0.3866 = g/L
mg/dL x 0.0259 = mmol/L

Limitations - interference[17]

Criterion: Recovery within ± 10 % of initial values at a cholesterol concentration of < 5.2 mmol/L (< 200 mg/dL).

Icterus: No significant interference up to an I index of 14 for conjugated bilirubin (approximate conjugated bilirubin concentration: 239 µmol/L (14 mg/dL)).
No significant interference up to an I index of 7 for unconjugated bilirubin (approximate unconjugated bilirubin concentration: 120 µmol/L (7 mg/dL)).

Hemolysis: No significant interference up to an H index of 350 (approximate hemoglobin concentration: 217 µmol/L (350 mg/dL)).

Lipemia (Intralipid): No significant interference up to an L index of 1000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[18,19]

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Special wash requirements

No interfering assays are known which require special wash steps.

Limits and ranges

Measuring range

0.25-20.7 mmol/L (9.7-800 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:10 dilution.
Results from samples diluted by the rerun function are automatically multiplied by a factor of 10.

Lower limits of measurement

Lower detection limit of the test

0.25 mmol/L (9.7 mg/dL)
The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Expected values

Clinical interpretation according to the recommendations of the European Atherosclerosis Society:[20]

	mmol/L	*mg/dL*	*Lipid metabolic disorder*
Cholesterol Triglycerides	< 5.2 < 2.3	(< 200) (< 200)	No
Cholesterol	5.2-7.8	(200-300)	Yes, if HDL-cholesterol < 0.9 mmol/L (< 35 mg/dL)
Cholesterol Triglycerides	> 7.8 > 2.3	(> 300) (> 200)	Yes

Recommendations of the NCEP Adult Treatment Panel for the following risk-cutoff thresholds for the US American population:[21]

Desirable cholesterol level	< 5.2 mmol/L	(< 200 mg/dL)
Borderline high cholesterol	5.2-6.2 mmol/L	(200-240 mg/dL)
High cholesterol	≥ 6.2 mmol/L	(≥ 240 mg/dL)

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below.
Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol. Repeatability n = 21, intermediate precision (3 aliquots per run, 1 run per day, 10 days).
* repeatability = within-run precision
** intermediate precision = total precision / between run precision / between day precision
The following results were obtained:

Repeatability	*Mean* *mmol/L (mg/dL)*	*SD* *mmol/L (mg/dL)*	*CV* *%*
Precinorm U	2.4 (92.8)	0.01 (0.39)	0.45
Precipath U	4.8 (185.6)	0.03 (1.16)	0.69
Human serum 1	3.0 (116.0)	0.05 (1.93)	1.72
Human serum 2	8.1 (313.1)	0.06 (2.32)	0.73

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CHOL2

Cholesterol Gen.2

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Intermediate precision	Mean mmol/L (mg/dL)	SD mmol/L (mg/dL)	CV %
Precinorm U	2.42 (93.6)	0.025 (0.97)	1.01
Precipath U	4.90 (189.4)	0.054 (2.09)	1.10
Human serum 3	3.40 (131.4)	0.046 (1.82)	1.37
Human serum 4	10.9 (421.4)	0.156 (6.03)	1.43

Method comparison

Cholesterol values for human serum and plasma samples obtained on the **cobas c** 111 analyzer (y) were compared to those determined with the same reagent on a COBAS INTEGRA 400 analyzer (x).

Sample size (n) = 111

Passing/Bablok[22]	Linear regression
y = 1.019x - 0.010 mmol/L	y = 0.984x + 0.115 mmol/L
r = 0.973	r = 0.998

The sample concentrations were between 0.46 and 18.95 mmol/L (17.8 and 732.6 mg/dL).

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APPENDIX M

CRP

05078799001V1

CRPHS

C-Reactive Protein (Latex) High Sensitive Assay

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• Indicates **cobas c** systems on which reagents can be used

Order Information			**cobas c** systems
			cobas c 111
C-Reactive Protein (Latex) High Sensitive Assay			
4 x 50 tests	Cat. No. **05007402** 190	CRPHS: ACN 217	•
Calibrator f.a.s. Proteins (5 x 1 mL)	Cat. No. **11355279** 216	Code 656	
Calibrator f.a.s. Proteins (5 x 1 mL, for USA)	Cat. No. **11355279** 160	Code 656	
CRP T Control N (5 x 0.5 mL)	Cat. No. **20766321** 322	Code 235	
Precinorm Protein (3 x 1 mL)	Cat. No. **10557897** 122	Code 302	
NaCl Diluent 9% (4 x 12 mL)	Cat. No. **04774230** 190	Code 951	

English

System Information
CRPHS: ACN 217

Intended use
In vitro test for the quantitative determination of C-reactive protein (CRP) in human serum and plasma on the **cobas c** 111 system. Measurement of CRP is of use for the detection and evaluation of inflammatory disorders and associated diseases, infection and tissue injury. Highly sensitive measurement of CRP may also be used as an aid in the assessment of the risk of future coronary heart disease. When used as an adjunct to other laboratory evaluation methods of acute coronary syndromes, it may also be an additional independent indicator of recurrent event prognosis in patients with stable coronary disease or acute coronary syndrome.

Summary[1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21]
C-reactive protein is the classic acute phase protein in inflammatory reactions. It is synthesized by the liver and consists of five identical polypeptide chains that form a five-member ring having a molecular weight of 105000 Daltons. CRP is the most sensitive of the acute phase reactants and its concentration increases rapidly during inflammatory processes. Complexed CRP activates the complement system beginning with C1q. CRP then initiates opsonization and phagocytosis of invading cells, but its main function is to bind and detoxify endogenous toxic substances produced as a result of tissue damage.

CRP assays are used to detect systemic inflammatory processes (apart from certain types of inflammation such as SLE and Colitis ulcerosa); to assess treatment of bacterial infections with antibiotics; to detect intrauterine infections with concomitant premature amniorrhexis; to differentiate between active and inactive forms of disease with concurrent infection, e.g. in patients suffering from SLE or Colitis ulcerosa; to therapeutically monitor rheumatic disease and assess anti-inflammatory therapy; to determine the presence of post-operative complications at an early stage, such as infected wounds, thrombosis and pneumonia, and to distinguish between infection and bone marrow transplant rejection.

Sensitive CRP measurements have been used and discussed for early detection of infection in pediatrics and risk assessment of coronary heart disease. Several studies came to the conclusion that the highly sensitive measurement of CRP could be used as a marker to predict the risk of coronary heart disease in apparently healthy persons and as an indicator of recurrent event prognosis. Increases in CRP values are non-specific and should not be interpreted without a complete clinical history. The American Heart Association and the Centers for Disease Control and Prevention have made several recommendations concerning the use of high sensitivity C-Reactive Protein (hsCRP) in cardiovascular risk assessment. Testing for any risk assessment should not be performed while there is an indication of infection, systemic inflammation or trauma. Patients with persistently unexplained hsCRP levels above 10 mg/L (95.2 nmol/L) should be evaluated for non-cardiovascular etiologies. When using hsCRP to assess the risk of coronary heart disease, measurements should be made on metabolically stable patients and compared to previous values. Optimally, the average of hsCRP results repeated two weeks apart should be used for risk assessment. Screening the entire adult population for hsCRP is not recommended, and hsCRP is not a substitute for traditional cardiovascular risk factors. Acute coronary syndrome management should not depend solely on hsCRP measurements. Similarly, application of secondary prevention measures should be based on global risk assessment and not solely on hsCRP measurements. Serial measurements of hsCRP should not be used to monitor treatment.

Various assay methods are available for CRP determination, such as nephelometry and turbidimetry. The Roche CRP assay is based on the principle of particle-enhanced immunological agglutination.

Test principle[22,23]
Particle enhanced immuno-turbidimetric assay.
Human CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies. The precipitate is determined turbidimetrically.

Reagents - working solutions

R1 TRIS buffer with bovine serum albumin and immunoglobulins (mouse); preservative; stabilizers

SR Latex particles coated with anti-CRP (mouse) in glycine buffer; preservative; stabilizers

Precautions and warnings
For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Reagent handling

R1 Ready for use.

SR Ready for use. Before use, invert several times, avoiding the formation of foam.

Storage and stability

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Shelf life at 2-8°C:	See expiration date on reagent
On-board in use and refrigerated on the analyzer:	4 weeks

NaCl Diluent 9%

Shelf life at 2-8°C:	See expiration date on reagent
On-board in use and refrigerated on the analyzer:	4 weeks

Specimen collection and preparation
For specimen collection and preparation, only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.

Serum.
Plasma: Li-heparin, K_2-EDTA plasma.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability:[24] 3 days at 15-25°C
8 days at 2-8°C
3 years at (-15)-(-25)°C

Materials provided
See "Reagents - working solutions" section for reagents.

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Materials required (but not provided)
See "Order information" section.
Distilled water
General laboratory equipment

Assay
For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

cobas c 111 system – test definition

Measuring mode	Absorbance
Abs. calculation mode	Kinetic
Reaction direction	Increase
Wavelength A	552 nm
Calc. first/last	17/34
Unit	mg/L (nmol/L, mg/dL)
Reaction mode	R1-S-SR

Pipetting parameters

		Diluent (H_2O)
R1	82 µL	
Sample	6 µL	48 µL
SR	28 µL	14 µL
Total volume	178 µL	

Calibration

Calibrator	Calibrator f.a.s. Proteins
Calibration dilution ratio	1:5, 1:10, 1:20, 1:40, 1:80, performed automatically by the instrument, and Standard 6 = 0 mg/L.
Calibration mode	Linear Interpolation
Calibration interval	Each lot and as required following quality control procedures

Enter the assigned lot-specific CRPHS value of the undiluted calibrator (mg/L), indicated in the package insert of C.f.a.s. Proteins.
Traceability: This method has been standardized against the reference preparation of the IRMM (Institute for Reference Materials and Measurements) BCR470/CRM470 (RPPHS - Reference Preparation for Proteins in Human Serum).[25]

Quality control
For quality control, use control materials as listed in the "Order information" section.
Other suitable control material can be used in addition.
The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

Calculation
The **cobas c** 111 analyzer automatically calculates the analyte concentration of each sample.

Conversion factors: mg/L x 9.52 = nmol/L
mg/L x 0.1 = mg/dL

Limitations - interference[26]
Criterion: Recovery within ±10% of initial values at CRP levels of 3.0 mg/L.
Icterus: No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).
Hemolysis: No significant interference up to an H index of 700 (approximate hemoglobin concentration: 435 µmol/L (700 mg/dL)).
Lipemia (Intralipid): No significant interference up to an L index of 500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.
Rheumatoid factors up to 1200 IU/mL do not interfere.
High-dose hook effect: does not occur at CRP concentrations below 40 mg/L or 380 nmol/L. Samples with concentrations >40 mg/L are flagged either >TEST RNG or "HIGH ACT".
Drugs: No interference was found at therapeutic concentrations using common drug panels.[27]
In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.
Although measures were taken to minimize interference caused by human anti-mouse antibodies, erroneous findings may be obtained from samples taken from patients who have been treated with monoclonal mouse antibodies or have received them for diagnostic purposes.
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.
Special wash requirements
No interfering assays are known which require special wash steps.

Measuring range
0.15-20.0 mg/L (1.43-190 nmol/L, 0.015-2.0 mg/dL)
Lower detection limit
0.15 mg/L (1.43 nmol/L, 0.015 mg/dL)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard.

Functional sensitivity (limit of quantitation)
0.3 mg/L (2.86 nmol/L)
The functional sensitivity (limit of quantitation) is the lowest CRP concentration that can be reproducibly measured with an inter-assay coefficient of variation <10%.

Expected values
Consensus reference interval for adults:[28]

IFCC/CRM 470

mg/dL	mg/L	nmol/L
<0.5	<5.0	<47.6

The CDC/AHA recommended the following hsCRP cut-off points (tertiles) for CVD risk assessment:[21,29]

hsCRP level (mg/L)	hsCRP level (nmol/L)	Relative risk
<1.0	<9.52	low
1.0-3.0	9.52-28.6	average
>3.0	>28.6	high

Patients with higher hsCRP concentrations are more likely to develop myocardial infarction and severe peripheral vascular disease.
5-95% reference intervals of neonates and children:[30]
Neonates (0-3 weeks): 0.1-4.1 mg/L (0.95-39.0 nmol/L)
Children (2 months-15 years): 0.1-2.8 mg/L (0.95-26.7 nmol/L)
It is important to monitor the CRP concentration during the acute phase of the illness.
Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.
Increases in CRP values are non-specific and should not be interpreted without a complete clinical history.
When using hsCRP to assess the risk of coronary heart disease, measurements should be made on metabolically stable patients and compared to previous values. Optimally, the average of hsCRP results repeated two weeks apart should be used for risk assessment. Measurements should

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be compared to previous values. When the results are being used for risk assessment, patients with persistently unexplained hsCRP levels of above 10 mg/L (95.2 nmol/L) should be evaluated for non-cardiovascular origins. Testing for any risk assessment should not be performed while there is indication of infection, systemic inflammation or trauma.[21]

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 30).
The following results were obtained:

Within-run	*Mean*	*SD*	*CV*
	mg/L (nmol/L, mg/dL)	*mg/L (nmol/L)*	*%*
Precinorm Protein	11.4 (109, 1.14)	0.044 (0.421)	0.39
CRP T Control N	4.06 (38.7, 0.406)	0.014 (0.133)	0.34
Human serum 1	0.49 (4.66, 0.049)	0.007 (0.069)	1.46
Human serum 2	4.02 (38.3, 0.402)	0.024 (0.232)	0.61
Human serum 3	16.9 (161, 1.69)	0.051 (0.488)	0.30

Total	*Mean*	*SD*	*CV*
	mg/L (nmol/L, mg/dL)	*mg/L (nmol/L)*	*%*
Precinorm Protein	11.3 (108, 1.13)	0.057 (0.543)	0.51
CRP T Control N	3.90 (37.1, 0.39)	0.038 (0.362)	0.97
Human serum 4	0.48 (4.57, 0.048)	0.010 (0.093)	2.04
Human serum 5	3.91 (37.2, 0.39)	0.054 (0.514)	1.37
Human serum 6	16.8 (160, 1.68)	0.116 (1.104)	0.69

Method comparison

CRP values for human serum and plasma samples obtained on the **cobas c 111** analyzer (y) were compared to those determined with the same reagent on a COBAS INTEGRA 400 analyzer (x).

Sample size (n) = 79

Passing/Bablok[31]	Linear regression
y = 1.035x – 0.111 mg/L	y = 1.051x – 0.202 mg/L
r= 0.962	r= 0.999

The sample concentrations of the reference system (x) were between 0.21 and 18.6 mg/L (2.0 and 177 nmol/L, 0.021 and 1.86 mg/dL).

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APPENDIX N

GLUCOSE

04773365001V3

GLUC2

Glucose HK

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• Indicates **cobas c** systems on which reagents can be used

Order information			**cobas c** systems
			cobas c 111
Glucose HK			
4 x 100 tests	Cat. No. **04657527** 190	GLU2: ACN 767 GLU2U: ACN 305	•
Calibrator f.a.s. (12 x 3 mL)	Cat. No. **10759350** 190	Code 401	
Calibrator f.a.s. (12 x 3 mL, for USA)	Cat. No. **10759350** 360	Code 401	
Precinorm U plus, (10 x 3 mL)	Cat. No. **12149435** 122	Code 300	
Precinorm U plus, (10 x 3 mL, for USA)	Cat. No. **12149435** 160	Code 300	
Precipath U plus (10 x 3 mL)	Cat. No. **12149443** 122	Code 301	
Precipath U plus (10 x 3 mL, for USA)	Cat. No. **12149443** 160	Code 301	
Precinorm U (20 x 5 mL)	Cat. No. **10171743** 122	Code 300	
Precipath U (20 x 5 mL)	Cat. No. **10171778** 122	Code 301	

English

System information
GLU2: ACN 767
GLU2U: ACN 305

Intended use
In vitro test for the quantitative determination of glucose in human serum, plasma and urine on the **cobas c** 111 system.

Summary[1,2,3]
Glucose is the major carbohydrate present in the peripheral blood. Oxidation of glucose is the major source of cellular energy in the body. Glucose derived from dietary sources is converted to glycogen for storage in the liver or to fatty acids for storage in adipose tissue. The concentration of glucose in blood is controlled within narrow limits by many hormones, the most important of which are produced by the pancreas.

The most frequent cause of hyperglycemia is diabetes mellitus resulting from a deficiency in insulin secretion or action. A number of secondary factors also contribute to elevated blood glucose levels. These include pancreatitis, thyroid dysfunction, renal failure and liver disease.

Hypoglycemia is less frequently observed. A variety of conditions may cause low blood glucose levels such as insulinoma, hypopituitarism or insulin induced hypoglycemia.

Glucose measurement in urine is used as a diabetes screening procedure and to aid in the evaluation of glucosuria, to detect renal tubular defects, and in the management of diabetes mellitus.

Test principle
UV test
Enzymatic reference method with hexokinase.[4,5]
Hexokinase catalyzes the phosphorylation of glucose to glucose-6-phosphate by ATP.

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{G-6-P} + \text{ADP}$$

Glucose-6-phosphate dehydrogenase oxidizes glucose-6-phosphate in the presence of NADP to gluconate-6-phosphate. No other carbohydrate is oxidized. The rate of NADPH formation during the reaction is directly proportional to the glucose concentration and is measured photometrically.

$$\text{G-6-P} + \text{NADP}^+ \xrightarrow{\text{G-6-PDH}} \text{gluconate-6-P} + \text{NADPH} + \text{H}^+$$

Reagents - working solutions
R1 TRIS buffer: 100 mmol/L, pH 7.8; Mg^{2+}: 4 mmol/L; ATP: ≥1.7 mmol/L; NADP: ≥1.0 mmol/L; preservative
SR HEPES buffer: 30 mmol/L, pH 7.0; Mg^{2+}: 4 mmol/L; HK (yeast): ≥130 µkat/L; G-6-PDH (*E. coli*): ≥250 µkat/L; preservative

Precautions and warnings
For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Reagent handling
Ready for use.

Storage and stability
GLUC2

Shelf life at 2-8°C:	See expiration date on reagent
On-board in use and refrigerated on the analyzer:	4 weeks

Specimen collection and preparation
For specimen collection and preparation, only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.
Serum
Plasma: Li-heparin, K_3-EDTA or Na-fluoride plasma.
Collect blood by venipuncture from fasting individuals using an evacuated tube system. The stability of glucose in specimens is affected by storage temperature, bacterial contamination, and glycolysis. Plasma or serum samples without preservative should be separated from the cells or clot within half an hour of being drawn. When blood is drawn and permitted to clot and to stand uncentrifuged at room temperature, the average decrease in serum glucose is ~7% in 1 hour (0.28 to 0.56 mmol/L or 5 to 10 mg/dL). This decrease is the result of glycolysis. Glycolysis can be inhibited by collecting the specimen in fluoride tubes.[1]
The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Stability (no hemolysis):[5]	8 hours at 15-25°C 72 hours at 2-8°C
Stability in Na-fluoride plasma:[5]	24 hours at 20-25°C

Urine
Collect urine in a dark bottle. For 24 hours urine collections, glucose may be preserved by adding 5 mL of glacial acetic acid to the container before collection. Unpreserved urine samples may lose up to 40% of their glucose after 24-hours storage at room temperature.[3] Therefore, keep samples on ice during collection.[5]
Centrifuge samples containing precipitates before performing the assay.

Materials provided
See "Reagents - working solutions" section for reagents.

Materials required (but not provided)
See "Order information" section
Deionized water
General laboratory equipment

Assay
For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.

GLUC2

Glucose HK

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The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum, plasma and urine

cobas c 111 system – test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction direction	Increase
Wavelength A/B	340/409 nm
Calc. first/last (serum, plasma)	16/37
Calc. first/last (urine)	16/38
Unit	mmol/L
Reaction mode	R1-S-SR

Pipetting parameters

		Diluent (H_2O)
R1	150 µL	
Sample	2 µL	20 µL
SR	30 µL	
Total volume	202 µL	

Calibration

Calibrators	Calibrator f.a.s. Deionized water is used automatically by the instrument as the zero calibrator
Calibration mode	Linear regression
Calibration interval	Each lot and as required following quality control procedures.

Traceability: This method has been standardized against ID/MS.

Quality control

Serum/plasma

For quality control, use control materials as listed in the "Order information" section.

Other suitable control material can be used in addition.

Urine

Quantitative urine controls are recommended for routine quality control.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The **cobas c** 111 analyzer automatically calculates the analyte concentration of each sample.

Conversion factors: mmol/L x 18.02 = mg/dL
mmol/L x 0.1802 = g/L
mg/dL x 0.0555 = mmol/L

Limitations - interference[6]

Criterion: Recovery within ±10% of initial value at a glucose concentration of 6.38 mmol/L (115 mg/dL).

Serum, plasma

Icterus: No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).

Hemolysis: No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L (1000 mg/dL)).

Lipemia (Intralipid): No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found using common drug panels.[7]

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

NOTE: Glucose values achieved on some proficiency testing materials, when evaluated against a glucose oxidase-oxygen electrode comparison method, demonstrate an approximate 3% positive bias on average.

Special wash requirements

No interfering assays are known which require special wash steps

Measuring range

Serum, plasma and urine

0.11-40 mmol/L (1.98-720 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:10 dilution. Results from samples diluted by the rerun function are automatically multiplied by a factor of 10.

Lower detection limit

0.11 mmol/L (1.98 mg/dL)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard.

Expected values

Plasma[8]		
Fasting	3.88-6.38 mmol/L	(70-115 mg/dL)
Urine[9]		
1st morning urine	0.3-1.1 mmol/L	(6-20 mg/dL)
24 hours urine	0.3-0.96 mmol/L	(6-17 mg/dL)
acc. to Tietz:[5]		
Serum/plasma		
Adults	4.11-5.89 mmol/L	(74-106 mg/dL)
60-90 years	4.56-6.38 mmol/L	(82-115 mg/dL)
>90 years	4.16-6.72 mmol/L	(75-121 mg/dL)
Children	3.33-5.55 mmol/L	(60-100 mg/dL)
Neonates (1 day)	2.22-3.33 mmol/L	(40-60 mg/dL)
Neonates (>1 day)	2.78-4.44 mmol/L	(50-80 mg/dL)
Urine		
24 hours urine	<2.78 mmol/24 hours	(<0.5 g/24 hours)
Random urine	0.06-0.83 mmol/L	(1-15 mg/dL)

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 30).

The following results were obtained:

Serum, plasma

Within-run	*Mean* mmol/L (mg/dL)	*SD* mmol/L (mg/dL)	*CV* %
Precinorm U	5.03 (90.6)	0.05 (0.9)	1.0
Precipath U	14.0 (252)	0.1 (2)	0.5
Human serum 1	2.27 (40.9)	0.03 (0.5)	1.1
Human serum 2	10.0 (180)	0.1 (2)	0.8
Total	*Mean* mmol/L (mg/dL)	*SD* mmol/L (mg/dL)	*CV* %
Precinorm U	5.12 (92.3)	0.03 (0.5)	0.7
Precipath U	14.1 (254)	0.1 (2)	0.5
Human serum 1	2.52 (45.4)	0.01 (0.2)	0.5
Human serum 2	9.89 (178)	0.06 (1)	0.6

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GLUC2

Glucose HK

Urine

Within-run	*Mean mmol/L (mg/dL)*	*SD mmol/L (mg/dL)*	*CV %*
Control level 1	1.90 (34.2)	0.01 (0.18)	0.7
Control level 2	15.7 (283)	0.04 (0.72)	0.3
Urine sample 1	0.80 (14.4)	0.01 (0.18)	1.6
Urine sample 2	30.0 (541)	0.10 (1.80)	0.4

Method comparison

Glucose values for human serum, plasma and urine samples obtained on the **cobas c** 111 analyzer (y) were compared with those determined using the same reagent on a COBAS INTEGRA 400 analyzer (x).

Serum, plasma

Sample size (n) = 80

Passing/Bablok[10]	Linear regression
y = 1.02x – 0.009 mmol/L	y = 1.02x + 0.019 mmol/L
τ = 0.983	r = 1.000

The sample concentrations were between 2.2 and 29.8 mmol/L (39.6 and 537 mg/dL).

Urine

Sample size (n) = 54

Passing/Bablok[10]	Linear regression
y = 0.984x – 0.007 mmol/L	y = 0.986x – 0.047 mmol/L
τ = 0.991	r = 1.000

The sample concentrations were between 0.13 and 39.1 mmol/L (2.34 and 705 mg/dL).

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APPENDIX O

HDL

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HDLC3

HDL-Cholesterol plus 3rd generation

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• Indicates **cobas c** systems on which reagents can be used

Order information			cobas c systems
			cobas c 111
HDL-Cholesterol plus 3rd generation			
4 x 100 tests	Cat. No. **04657560** 190	HDLC3: ACN 435	•
Calibrator f.a.s. Lipids (3 x 1 mL)	Cat. No. **12172623** 122	Code 424	
Calibrator f.a.s. Lipids (3 x 1 mL, for USA)	Cat. No. **12172623** 160	Code 424	
Precinorm L (4 x 3 mL)	Cat. No. **10781827** 122	Code 304	
Precipath HDL/LDL-C (4 x 3 mL)	Cat. No. **11778552** 122	Code 319	
NaCl Diluent 9%	Cat. No. **04774230** 190	Code 951	

English

System information
HDLC3: ACN 435

Intended use
In vitro diagnostic test for the quantitative determination of the HDL-cholesterol concentration in human serum and plasma on the **cobas c** 111 system.

Summary[1]
High density lipoproteins (HDL) are responsible for the reverse transport of cholesterol from the peripheral cells to the liver. Here, cholesterol is transformed to bile acids which are excreted into the intestine via the biliary tract. Monitoring of HDL-cholesterol in serum is of clinical importance since an inverse correlation exists between serum HDL-cholesterol concentrations and the risk of atherosclerotic disease. Elevated HDL-cholesterol concentrations are protective against coronary heart disease, while reduced HDL-cholesterol concentrations, particularly in conjunction with elevated triglycerides, increase the cardiovascular risk.[1] Strategies have emerged to increase the level of HDL-cholesterol to treat cardiovascular disease.[2,3]

A variety of methods are available to determine HDL-cholesterol, including ultracentrifugation, electrophoresis, HPLC, precipitation-based methods and direct methods. Of these, the direct methods are used routinely. Several approaches for direct measurement of HDL-cholesterol in serum have been proposed, including the use of magnetically responsive particles as polyanion-metal combinations and the use of polyethylene glycol (PEG) with anti-apoprotein B and anti-apoprotein CIII antibodies.

This automated method for direct determination of HDL-cholesterol in serum and plasma uses PEG-modified enzymes and dextran sulfate. When cholesterol esterase and cholesterol oxidase enzymes are modified by PEG, they show selective catalytic activities toward lipoprotein fractions, with the reactivity increasing in the order:
LDL < VLDL ≈ chylomicrons < HDL.[4,5,6,7,8,9,10,11,12,13,14,15,16]

Non-fasting sample results are slightly lower than fasting results. Comparable non-fasting results were observed with the beta quantification method.[17,18,19] The Roche direct HDL-cholesterol assay meets the 1998 National Institutes of Health (NIH) / National Cholesterol Education Program (NCEP) goals for acceptable performance.[20] The results of this method correlate with those obtained by precipitation-based methods and also by an ultracentrifugation method.

Test principle[4,5]
Homogeneous enzymatic colorimetric test.
In the presence of magnesium ions, dextran sulfate selectively forms water-soluble complexes with LDL, VLDL and chylomicrons which are resistant to PEG-modified enzymes.
The cholesterol concentration of HDL-cholesterol is determined enzymatically by cholesterol esterase and cholesterol oxidase coupled with PEG to the amino groups (approx. 40%).

Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase.

$$\text{HDL-cholesterol esters} + H_2O \xrightarrow{\text{PEG-cholesterol esterase}} \text{HDL-cholesterol} + \text{RCOOH}$$

In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ^4-cholestenone and hydrogen peroxide.

$$\text{HDL-cholesterol} + O_2 \xrightarrow{\text{PEG-cholesterol oxidase}} \Delta^4\text{-cholestenone} + H_2O_2$$

In the presence of peroxidase (POD), the hydrogen peroxide generated reacts with 4-amino-antipyrine and HSDA to form a purple-blue dye. The color intensity of this dye is directly proportional to the HDL-cholesterol concentration and is measured photometrically.

$$2\,H_2O_2 + \text{4-amino-antipyrine} + \text{HSDA}^* + H^+ + H_2O \xrightarrow{\text{POD}} \text{purple-blue pigment} + 5\,H_2O$$

*HSDA = Sodium N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline

Reagents - working solutions

R1 HEPES buffer: 10.07 mmol/L; CHES: 96.95 mmol/L, pH 7.4; Dextran sulfate: 1.5 g/L; magnesium nitrate hexahydrate: ≥11.7 mmol/L; HSDA: 0.96 mmol/L; ascorbate oxidase (Eupenicillium sp., recombinant): >50 µkat/L; POD (horseradish): ≥16.7 µkat/L; preservative

SR HEPES buffer: 10.07 mmol/L, pH 7.0; PEG-cholesterol esterase (Pseudonomas spec.): ≥3.33 µkat/L; PEG-cholesterol oxidase (Streptomyces sp., recombinant): ≥127 µkat/L; POD (horseradish): ≥333 µkat/L; 4-amino-antipyrine: 2.46 mmol/L; preservative

Precautions and warnings
For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Reagent handling
Ready for use.
The intrinsic pink color of the cholesterol reagent does not interfere with the test.
Inaccurate pipetting of reagent, leading to potentially erroneous results, may be caused by excessive foaming of this reagent. Ensure that foam is removed from the surface of the reagent prior to setting the reagent in the analyzer.

Storage and stability

HDLC3	
Shelf life at 2-8°C:	See expiration date on reagent
On-board in use and refrigerated on the analyzer:	3 weeks
NaCl Diluent 9%	
Shelf life at 2-8°C:	See expiration date on reagent
On-board in use and refrigerated on the analyzer:	4 weeks

Specimen collection and preparation
For specimen collection and preparation, only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.
Serum.
Plasma: Li-Heparin, K_3-EDTA plasma.
EDTA plasma causes decreased results.[21]
It is reported, that EDTA stabilizes lipoproteins.[22]
Fasting and non-fasting samples can be used.[16] Collect blood by using an evacuated tube or syringe. Specimens should preferably be analyzed on the day of collection.

HDLC3

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The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability:[19] 7 days at 2-8°C
30 days at -70°C

Materials provided

See "Reagents - working solutions" section for reagents.

Materials required (but not provided)

See "Order information" section.
Deionized water
General laboratory equipment

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

cobas c 111 test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction direction	Increase
Wavelength A/B	583/659 nm
Calc. first/last	16/37
Unit	mmol/L
Reaction mode	R1-S-SR

Pipetting parameters

		Diluent (H_2O)
R1	150 µL	
Sample	2.5 µL	7.0 µL
SR	50 µL	
Total volume	209.5 µL	

Calibration

Calibrators	Calibrator f.a.s. Lipids Deionized water is used automatically by the instrument as the zero calibrator.
Calibration mode	Linear regression
Calibration interval	Each lot and as required following quality control procedures

Traceability:[19] This method has been standardized against the designated CDC reference method (designated comparison method).[20] The standardization meets the requirements of the "HDL Cholesterol Method Evaluation Protocol for Manufacturers" of the US National Reference System for Cholesterol, CRMLN (Cholesterol Reference Method Laboratory Network), November 1994.

Quality control

For quality control, use control materials as listed in the "Order information" section.
Other suitable control material can be used in addition.
The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.
Follow the applicable government regulations and local guidelines for quality control.

Quality control materials are intended for use only as monitors of accuracy and precision. The Laboratory Standardization Panel (LSP) of the National Cholesterol Education Program in the United States recommends two levels of controls, one in the normal range (0.9-1.7 mmol/L or 35-65 mg/dL) and one near the concentration for decision making (<0.9 mmol/L or <35 mg/dL).

Calculation

The **cobas c** 111 analyzer automatically calculates the analyte concentration of each sample.

Conversion factors: mmol/L x 38.66 = mg/dL
mmol/L x 0.3866 = g/L
mg/dL x 0.0259 = mmol/L

Limitations - interference[24,25]

Criterion: Recovery within ±10% of initial value at a HDL-cholesterol concentration of 1.6 mmol/L (61.8 mg/dL).

Icterus: No significant interference up to an I index of 28 for conjugated and 60 for unconjugated bilirubin (approximate conjugated bilirubin concentration 478 µmol/L (28 mg/dL) and approximate unconjugated bilirubin concentration 1026 µmol/L (60 mg/dL)).

Hemolysis: No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L (1000 mg/dL)).

Lipemia: No significant interference up to an L index of 1000. No significant interference from native triglycerides up to 11.4 mmol/L (1000 mg/dL). There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

The claim for lipemia interference is based on the Glick model, which uses Intralipid as an artificial substrate. To date, there is no model available which can mimic interference by triglycerides, as triglyceride levels in patient specimens behave unpredictably, depending on the nature of the esterified fatty acids in the samples. Patient specimens with elevated triglyceride levels are very often lipemic. Therefore customers cannot verify interference by triglycerides in patient specimens.

Other: Elevated concentrations of free fatty acids and denatured proteins may cause falsely elevated HDL-cholesterol results.
In rare cases, elevated immunoglobulin concentrations can lead to falsely decreased HDL-cholesterol results.
Ascorbic acid up to 2.84 mmol/L (50 mg/dL) does not interfere.

Abnormal liver function affects lipid metabolism; consequently, HDL and LDL results are of limited diagnostic value. In some patients with abnormal liver function, the HDL-cholesterol results may significantly differ from those obtained using acknowledged reference methods such as ultracentrifugation or the DCM (designated comparison method).

Drugs: No interference was found using common drug panels.[26]

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Special wash requirements

No interfering assays are known which require special wash steps

Measuring range

0.08-3.12 mmol/L (3-120 mg/dL)

Determine samples having higher concentrations via the rerun function.
Dilution of samples via the rerun function is a 1:4 dilution.
Results from samples diluted by the rerun function are automatically multiplied by a factor of 4.

Lower detection limit

0.08 mmol/L (3 mg/dL)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard.

Expected values

	No risk	Moderate risk	High risk
Females[27,28,29]	>1.68 mmol/L (>65 mg/dL)	1.15-1.68 mmol/L (45-65 mg/dL)	<1.15 mmol/L (<45 mg/dL)
Males[27,28,29]	>1.45 mmol/L (>55 mg/dL)	0.90-1.45 mmol/L (35-55 mg/dL)	<0.90 mmol/L (<35 mg/dL)

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National Cholesterol Education Program (NCEP) guidelines:[30]
<40 mg/dL: Low HDL-cholesterol (major risk factor for CHD)
≥60 mg/dL: High HDL-cholesterol ("negative" risk factor for CHD)
HDL-cholesterol is affected by a number of factors, e.g. smoking, exercise, hormones, sex and age.
Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

National Cholesterol Education Program (NCEP) guidelines are based on serum values, and when classifying patients, serum or serum equivalent values should be used. Therefore the NCEP recommends a factor of 1.03 to convert EDTA plasma values to serum values. However, our own investigations revealed that a factor of 1.06 should be used for the HDLC3 reagent. To comply with the 1998 NCEP goal of a <5% bias we recommend that each laboratory validate this conversion factor and enter it into the test parameters for HDL_C (435).[23]

Specific performance data
Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision
Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 30). The following results were obtained:

Within-run	*Mean mmol/L (mg/dL)*	*SD mmol/L (mg/dL)*	*CV %*
Precinorm L	1.20 (46.4)	0.008 (0.309)	0.67
Precipath HDL/LDL-C	0.92 (35.6)	0.009 (0.363)	1.03
Human serum 1	0.85 (32.9)	0.013 (0.503)	1.53
Human serum 2	2.46 (95.1)	0.021 (0.812)	0.85
Total	*Mean mmol/L (mg/dL)*	*SD mmol/L (mg/dL)*	*CV %*
Precinorm L	1.23 (47.6)	0.015 (0.580)	1.21
Precipath HDL/LDL-C	0.71 (27.4)	0.011 (0.421)	1.54
Human serum 3	0.51 (19.7)	0.013 (0.491)	2.47
Human serum 4	2.09 (80.8)	0.016 (0.615)	0.76

Method comparison
HDL-cholesterol values for human serum and plasma samples obtained on the **cobas c** 111 analyzer (y) were compared with those determined using the same reagent on a COBAS INTEGRA 400 analyzer (x).

Sample size (n) = 101

Passing/Bablok[31]	Linear regression
y = 0.970x + 0.015 mmol/L	y = 0.970x + 0.019 mmol/L
r = 0.977	r = 0.999

The sample concentrations were between 0.16 and 3.05 mmol/L (6.2 and 118 mg/dL).

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APPENDIX P

TRIGLYCERIDES

TRIGL

Triglycerides

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• Indicates **cobas c** systems on which reagents can be used

Order information

			cobas c systems
			cobas c 111
Triglycerides			
4 x 50 tests	Cat. No. **04657594** 190	TRIGL: ACN 781	•
Calibrator f.a.s. (12 x 3 mL)	Cat. No. **10759350** 190	Code 401	
Calibrator f.a.s. (12 x 3 mL, for USA)	Cat. No. **10759350** 360	Code 401	
Precinorm U plus (10 x 3 mL)	Cat. No. **12149435** 122	Code 300	
Precinorm U plus (10 x 3 mL, for USA)	Cat. No. **12149435** 160	Code 300	
Precipath U plus (10 x 3 mL)	Cat. No. **12149443** 122	Code 301	
Precipath U plus (10 x 3 mL, for USA)	Cat. No. **12149443** 160	Code 301	
Precinorm U (20 x 5 mL)	Cat. No. **10171743** 122	Code 300	
Precipath U (20 x 5 mL)	Cat. No. **10171778** 122	Code 301	

English

System information
TRIGL: ACN 781

Intended use
In vitro test for the quantitative determination of triglycerides in human serum and plasma on the **cobas c** 111 system.

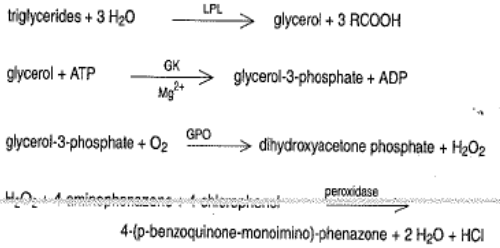
Summary[1,2,3,4,5,6]
Triglycerides are esters of the trihydric alcohol glycerol with 3 long-chain fatty acids. They are partly synthesized in the liver and partly ingested in food.

The determination of triglycerides is utilized in the diagnosis and treatment of patients having diabetes mellitus, nephrosis, liver obstruction, lipid metabolism disorders and numerous other endocrine diseases.

The enzymatic triglycerides assay as described by Eggstein and Kreutz still required saponification with potassium hydroxide. Numerous attempts were subsequently made to replace alkaline saponification by enzymatic hydrolysis with lipase. Bucolo and David tested a lipase/protease mixture; Wahlefeld used an esterase from the liver in combination with a particularly effective lipase from Rhizopus arrhizus for hydrolysis.

This method is based on the work by Wahlefeld using a lipoprotein lipase from microorganisms for the rapid and complete hydrolysis of triglycerides to glycerol followed by oxidation to dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide produced then reacts with 4-aminophenazone and 4-chlorophenol under the catalytic action of peroxidase to form a red dyestuff (Trinder endpoint reaction). The color intensity of the red dyestuff formed is directly proportional to the triglyceride concentration and can be measured photometrically.

Test principle[6]
Enzymatic colorimetric test.



Reagents - working solutions

R1 PIPES buffer: 50 mmol/L, pH 6.8; Mg^{2+}: 40 mmol/L; sodium cholate: 0.20 mmol/L; ATP: ≥1.4 mmol/L; 4-aminophenazone: ≥0.13 mmol/L; 4-chlorophenol: 4.7 mmol/L; LPL (Pseudomonas spec.): ≥83 µkat/L; GK (Bacillus stearothermophilus): ≥3 µkat/L; GPO (E. coli): ≥41 µkat/L; POD (horseradish): ≥1.6 µkat/L; preservative

Precautions and warnings
For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Reagent handling
Ready for use.
Inaccurate pipetting of reagent, leading to potentially erroneous results, may be caused by excessive foaming of this reagent. Ensure that foam is removed from the surface of the reagent prior to setting the reagent in the analyzer.

Storage and stability

TRIGL

Shelf life at 2-8°C:	See expiration date on reagent
On-board in use and refrigerated on the analyzer:	2 weeks

Specimen collection and preparation
For specimen collection and preparation, only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.

Serum
Plasma: Li-heparin, K_3-EDTA plasma.
EDTA tubes that are less than 1/2 full may cause a negative bias for triglycerides results.
Patients should refrain from eating for 10 to 14 hours before blood is drawn.
Samples must be drawn in a soap and glycerol free collection device.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability:[7] 5-7 days at 2-8°C
3 months at (-15)-(-25)°C
several years at (-60)-(-80)°C

Materials provided
See "Reagents - working solutions" section for reagents.

Materials required (but not provided)
See "Order information" section.
Deionized water
General laboratory equipment

Assay
For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

TRIGL

Triglycerides

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Application for serum and plasma

cobas c 111 system – test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction direction	Increase
Wavelength A/B	512/659 nm
Calc. first/last	6/21
Unit	mmol/L
Reaction mode	R-S

Pipetting parameters

		Diluent (H_2O)
R	120 µL	
Sample	2 µL	28 µL
Total volume	150 µL	

Calibration

Calibrator	Calibrator f.a.s. Deionized water is used automatically by the instrument as the zero calibrator
Calibration mode	Linear regression
Calibration interval	Each lot and as required following quality control procedures

Traceability: This method has been standardized against the ID/MS method.

Quality control

For quality control, use control materials as listed in the "Order information" section.
Other suitable control material can be used in addition.
The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

Calculation

The **cobas c** 111 analyzer automatically calculates the analyte concentration of each sample.

Conversion factors: mmol/L x 88.5 = mg/dL
mg/dL x 0.0113 = mmol/L

Limitations - Interference[9]

Criterion: Recovery within ± 10% of initial values at triglycerides levels of <2.3 mmol/L (<200 mg/dL).

Icterus: No significant interference up to an I index of 5 (approximate conjugated and unconjugated bilirubin concentration: 57 µmol/L (5 mg/dL)).

Hemolysis: No significant interference up to an H index of 200 (approximate hemoglobin concentration: 2124 µmol/L (200 mg/dL)).
Endogenous unesterified glycerol in the sample will falsely elevate serum triglycerides.

Drugs: No interference was found using common drug panels.[9]
Exception: Ascorbic acid and calcium dobesilate cause artificially low triglycerides results at the tested drug levels, levodopa, methyldopa and phenylbutazone cause artificially low triglycerides results at a higher drug level and Intralipid causes artificially high triglycerides results at a higher drug level.

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Special wash requirements

No interfering assays are known which require special wash steps.

Measuring range

0.1-10 mmol/L (8.85-885 mg/dL)

Determine samples having higher concentrations via the rerun function.
Dilution of samples via the rerun function is a 1:10 dilution.
Results from samples diluted by the rerun function are automatically multiplied by a factor of 10.

Lower detection limit

0.1 mmol/L (8.85 mg/dL)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard.

Expected values according to NCEP[10]

Normal range: <2.3 mmol/L (<200 mg/dL).

Clinical interpretation according to the recommendations of the European Atherosclerosis Society:[11]

	mmol/L	mg/dL	Lipid metabolism disorder
Cholesterol	<5.2	<200	No
Triglycerides	<2.3	<200	
Cholesterol	5.2-7.8	200-300	Yes if HDL-cholesterol <0.9 mmol/L (<35 mg/dL)
Cholesterol	>7.8	>300	Yes
Triglycerides	>2.3	>200	

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below.
Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 30).
The following results were obtained:

Within-run	*Mean mmol/L (mg/dL)*	*SD mmol/L (mg/dL)*	*CV %*
Precinorm U	1.3 (115)	0.01 (0.67)	0.58
Precipath U	2.2 (195)	0.02 (1.47)	0.75
Human serum 1	1.7 (151)	0.02 (1.96)	1.33
Human serum 2	5.9 (522)	0.05 (4.26)	0.82
Total	*Mean mmol/L (mg/dL)*	*SD mmol/L (mg/dL)*	*CV %*
Precinorm U	1.36 (120)	0.02 (1.91)	1.58
Precipath U	2.34 (207)	0.04 (3.60)	1.74
Human serum 3	1.22 (108)	0.01 (0.99)	0.92
Human serum 4	8.64 (765)	0.31 (27.8)	3.64

Method comparison

Triglycerides values for human serum and plasma samples obtained on the **cobas c** 111 analyzer (y) were compared with those determined using the same reagent on a COBAS INTEGRA 400 analyzer (x).

Sample size (n) = 73

Passing/Bablok[12]	Linear regression
y = 1.035x – 0.017 mmol/L	y = 1.040x – 0.015 mmol/L
r = 0.976	r = 0.998

The sample concentrations were between 0.4 and 10 mmol/L (35 and 885 mg/dL).

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TRIGL

Triglycerides

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ferences

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APPENDIX Q

INSULIN

HUMAN INSULIN SPECIFIC RIA KIT
250 TUBES (Cat. # HI-14K)

I. INTENDED USE

Insulin is a polypeptide hormone secreted from beta cells of the pancreas. The primary function of Insulin is to control blood glucose levels through its biochemical actions on cellular glucose uptake, glycogenesis, lipogenesis, and glucose oxidation. Insulin secretion into the bloodstream is predominantly controlled by the level of glucose in plasma but is also influenced by other factors, such as neural influences, intestinal hormones, and other beta cell secretary hormones. The measurement of in-vivo insulin concentrations may aid in the diagnosis of conditions, such as nesidioblastosis, islet-cell tumors, and various insulin resistant conditions, such as diabetes mellitus. This Human Insulin Specific Kit is for the quantitative determination of Insulin in serum, plasma, and tissue culture media. This assay does not cross-react with Human Proinsulin (<0.2%) and therefore measures "true" insulin levels. It is a completely homologous assay since the antibody was raised against purified Human Insulin and both the standard and the tracer are prepared with Human Insulin.
For research purposes only.

II. PRINCIPLES OF PROCEDURE

In radioimmunoassay, a fixed concentration of labeled tracer antigen is incubated with a constant dilution of antiserum such that the concentration of antigen binding sites on the antibody is limited, for example, only 50% of the total tracer concentration may be bound by antibody. If unlabeled antigen is added to this system, there is competition between labeled tracer and unlabeled antigen for the limited and constant number of binding sites on the antibody. Thus, the amount of tracer bound to antibody will decrease as the concentration of unlabeled antigen increases. This can be measured after separating antibody-bound from free tracer and counting one or the other, or both fractions. A standard curve is set up with increasing concentrations of standard unlabeled antigen and from this curve the amount of antigen in unknown samples can be calculated. Thus, the four basic necessities for a radioimmunoassay system are: a specific antiserum to the antigen to be measured, the availability of a radioactive labeled form of the antigen, a method whereby antibody-bound tracer can be separated from the unbound tracer, and finally, an instrument to count radioactivity.

The Millipore Human Insulin assay utilizes ^{125}I-labeled Human Insulin and a Human Insulin antiserum to determine the level of insulin in serum, plasma or tissue culture media by the double antibody/PEG technique[1].

III. REAGENTS SUPPLIED

Each kit is sufficient to run 250 tubes and contains the following reagents.

A. Assay Buffer
0.05M Phosphosaline pH 7.4 containing 0.025M EDTA, 0.08% Sodium Azide, and 1% RIA Grade BSA
Quantity: 40 mL/vial
Preparation: Ready to use

B. Human Insulin Antibody
Guinea Pig anti-Human Insulin Specific antibody in Assay Buffer
Quantity: 26 mL/vial
Preparation: Ready to use

III. REAGENTS SUPPLIED (continued)

C. ^{125}I-Insulin
^{125}I-Insulin Label, HPLC purified (specific activity 367 µCi/µg)
Lyophilized for stability. Freshly iodinated label contains <5 µCi (185 kBq), calibrated to the 1st Monday of each month.
Quantity: 27 mL/vial upon hydration
Preparation: Contents Lyophilized. Hydrate with entire contents of Label Hydrating Buffer. Allow to set at room temperature for 30 minutes, with occasional gentle mixing.

D. Label Hydrating Buffer
Assay Buffer containing Normal Guinea Pig Serum as a carrier. Used to hydrate ^{125}I-Insulin.
Quantity: 27 mL/vial
Preparation: Ready to use

E. Human Insulin Standards
Purified Recombinant Human Insulin in Assay Buffer at the following concentration:
200 µU/mL
Quantity: 2 mL/vial
Preparation: Ready to use

F. Quality Controls 1 & 2
Purified Recombinant Human Insulin in Assay Buffer
Quantity: 1 mL/vial
Preparation: Ready to use

G. Precipitating Reagent
Goat anti-Guinea Pig IgG serum, 3% PEG and 0.05% Triton X-100 in 0.05M Phosphosaline, 0.025M EDTA,
0.08% Sodium Azide
Quantity: 260 mL/vial
Preparation: Ready to use; chill to 4°C.

IV. STORAGE AND STABILITY

Refrigerate all reagents between 2 and 8°C for short-term storage. For prolonged storage (>2 weeks), freeze at ≤ -20°C. Avoid multiple (>5) freeze/thaw cycles. Refer to date on bottle for expiration when stored at ≤ -20°C. Do not mix reagents from different kits unless they have the same lot number.

V. REAGENT PRECAUTIONS

A. Radioactive Materials
This radioactive material may be received, acquired, possessed and used only by research personnel or clinical laboratories for in-vitro research tests not involving internal or external administration of the material, or the radiation there from, to human beings or animals. Its receipt, acquisition, possession, use and transfer are subject to the regulations of the U. S. Nuclear Regulatory Commission (NRC) or of a State with which the Commission has entered into an agreement for the exercise of regulatory authority.

The following are suggested general rules for the safe use of radioactive material. The customer's Radiation Safety Officer is ultimately responsible for the safe handling and use of radioactive material.

V. REAGENT PRECAUTIONS (continued)

1. Wear appropriate personal devices at all times while in areas where radioactive materials are used or stored.
2. Wear laboratory coats, disposable gloves, and other protective clothing at all times.
3. Monitor hands, shoes, and clothing and immediate area surrounding the workstation for contamination after each procedure and before leaving the area.
4. Do not eat, drink, or smoke in any area where radioactive materials are stored or used.
5. Never pipette radioactive material by mouth.
6. Dispose of radioactive waste in accordance with NRC rules and regulations.
7. Avoid contaminating objects such as telephones, light switches, doorknobs, etc.
8. Use absorbent pads for containing and easily disposing of small amounts of contamination.
9. Wipe up all spills immediately and thoroughly and dispose of the contaminated materials as radioactive waste. Inform Radiation Safety Officer.

B. Sodium Azide

Sodium Azide has been added to all reagents as a preservative at a concentration of 0.08%. Although it is at a minimum concentration, Sodium Azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build up.

VI. MATERIALS REQUIRED BUT NOT PROVIDED

1. Borosilicate glass tubes, 12 x 75 mm. (NOTE: Polypropylene or polystyrene tubes may be used if the investigator finds that the pellet formation is acceptably stable in their system.)
2. 100 µL pipet with disposable tips
3. 100 µL & 1.0 mL repeating dispenser
4. Refrigerated swing bucket centrifuge capable of developing 2,000 - 3,000 xg. (Use of fixed-angle buckets is not recommended.)
5. Absorbent paper
6. Vortex mixer
7. Refrigerator
8. Gamma Counter

VII. SPECIMEN COLLECTION AND STORAGE

1. A maximum of 100 µL per assay tube of serum or plasma can be used, although, 50 µL per assay tube is adequate for most applications. Tissue culture and other media may also be used.
2. Care must be taken when using heparin as an anticoagulant, since an excess will provide falsely high values[2]. Use no more than 10 IU heparin per mL of blood collected.
3. Specimens can be stored at 4°C if they will be tested within 24 hours of collection. For longer storage, specimens should be stored at ≤ -20°C. Avoid multiple (>5) freeze/thaw cycles.
4. Avoid using samples with gross hemolysis or lipemia.

VIII. ASSAY PROCEDURE

Standard Preparation

Use care in opening the Standard vial.

Label six glass tubes 1, 2, 3, 4, 5, and 6. Add 1.0 mL Assay Buffer to each of the six tubes. Prepare serial dilutions by adding 1.0 mL of the 200uU/mL standard to tube 1, mix well and transfer 1.0 mL of tube 1 to tube 2, mix well and transfer 1.0 mL of tube 2 to tube 3, mix well and transfer 1.0 mL of tube 3 to tube 4, mix well and transfer 1.0 mL of tube 4 to tube 5, mix well and transfer 1.0 mL of tube 5 to tube 6, mix well.

Note: Do not use a Repeater pipette. Change tip for every dilution. Wet tip with Standard before dispensing. Unused portions of standard should be stored at ≤ -20°C. Avoid multiple freeze/thaw cycles.

Tube #	Standard Concentration	Volume of Assay Buffer to Add	Volume of Standard to Add
1	100 uU/mL	1.0 mL	1.0 mL of 200 uU/mL
2	50 uU/mL	1.0 mL	1.0 mL of 100 uU/mL
3	25 uU/mL	1.0 mL	1.0 mL of 50 uU/mL
4	12.5 uU/mL	1.0 mL	1.0 mL of 25 uU/mL
5	6.25 uU/mL	1.0 mL	1.0 mL of 12.5 uU/mL
6	3.125 uU/mL	1.0 mL	1.0 mL of 6.25 uU/mL

For optimal results, accurate pipetting and adherence to the protocol are recommended.

Assay Set-Up, Day One

1. Pipet 300 µL of Assay Buffer to the Non-Specific Binding (NSB) tubes (3-4), 200 µl to Reference (Bo) tubes (5-6), and 100 µl to tubes 7 through the end of the assay.
2. Pipet 100 µL of Standards and Quality Controls in duplicate (see flow chart).
3. Pipet 100 µL of each Sample in duplicate. (NOTE: Smaller volumes of sample may be used when insulin concentrations are anticipated to be elevated or when sample size is limited. Additional Assay Buffer should be added to compensate for the difference so that the volume is equivalent to 100 µL, e.g., when using 50 µL of sample, add 50 µL of Assay Buffer). Refer to Section IX for calculation modification.
4. Pipet 100 µL of hydrated ^{125}I-Insulin to all tubes. Important: For preparation, see Section III, Part C.
5. Pipet 100 µL of Human Insulin antibody to all tubes except Total Count tubes (1-2) and NSB tubes (3-4).
6. Vortex, cover, and incubate overnight (20-24 hours) at room temperature (22-25°C).

Day Two

7. Add 1.0 mL of cold (4°C) Precipitating Reagent to all tubes (except Total Count tubes).
8. Vortex and incubate 20 minutes at 4°C.
9. Centrifuge, 4°C, all tubes [except Total Count tubes (1-2)] for 20 minutes at 2,000-3,000 xg.

NOTE: If less than 2,000 xg is used or if slipped pellets have been observed in previous runs, the time of centrifugation must be increased to obtain a firm pellet (e.g., 40 minutes). Multiple centrifuge runs within an assay must be consistent.

Conversion of rpm to xg:
$xg = (1.12 \times 10^{-5})\ (r)\ (rpm)^2$
r = radial distance in cm (from axis of rotation to the bottom of the tube)
rpm = revolutions per minute

10. Immediately decant the supernate of all tubes except Total Count tubes (1-2), drain tubes for at least 15-60 seconds (be consistent between racks) and blot excess liquid from lip of tubes. NOTE: Invert tubes only one time. Pellets are fragile and slipping may occur.
11. Count all tubes in a gamma counter for 1 minute. Calculate the µU/mL of Human Insulin in unknown samples using automated data reduction procedures.

Assay Procedure Flow Chart

	Day One						Day Two		
Set-up	Step 1	Steps 2 & 3	Step 4	Step 5	Step 6	Step 7	Step 8	Steps 9-11
Tube Number	Add Assay Buffer	Add Standard/ QC Sample	Add I-125 Human Insulin Tracer	Add Human Insulin Antibody	Vortex, Cover, and Incubate 20-24 hrs at RT	Add Precipitating Reagent	Vortex, and Incubate 20 min. at 4°C	Centrifuge at 4°C for 20 min., Decant, and Count
,2	-	-	100 µl	-		-		
,4	300 µl	-	100 µl	-		1.0 mL		
,6	200 µl	-	100 µl	100 µl		1.0 mL		
,8	100 µl	100 µl of 3,125 µU/mL	100 µl	100 µl		1.0 mL		
10	100 µl	100 µl of 6.25 µU/mL	100 µl	100 µl		1.0 mL		
,12	100 µl	100 µl of 12.5 µU/mL	100 µl	100 µl		1.0 mL		
,14	100 µl	100 µl of 25 µU/mL	100 µl	100 µl		1.0 mL		
,16	100 µl	100 µl of 50 µU/mL	100 µl	100 µl		1.0 mL		
,18	100 µl	100 µl of 100 µU/mL	100 µl	100 µl		1.0 mL		
,20	100 µl	100 µl of 200 µU/mL	100 µl	100 µl		1.0 mL		
,22	100 µl	100 µl of QC 1	100 µl	100 µl		1.0 mL		
-24	100 µl	100 µl of QC 2	100 µl	100 µl		1.0 mL		
,26	100 µl	100 µl of unknown	100 µl	100 µl		1.0 mL		
-n	100 µl	100 µl of unknown	100 µl	100 µl		1.0 mL		

IX. CALCULATIONS

A. Explanation

The calculations for Human Insulin can be automatically performed by most gamma counters possessing data reduction capabilities or by independent treatment of the raw data using a commercially available software package[3]. Choose weighted 4-parameter or weighted log/logit for the mathematical treatment of the data.

NOTE: Be certain the procedure used subtracts the NSB counts from each average count, except Total Counts, prior to final data reduction.

B. Manual Calculation

1. Average duplicate counts for Total Count tubes (1-2), NSB tubes (3-4), Total Binding tubes (reference, Bo) (5-6), and all duplicate tubes for standards and samples to the end of the assay.

2. Subtract the average NSB counts from each average count (except for Total Counts). These counts are used in the following calculations.

3. Calculate the percentage of tracer bound.
   (Total Binding Counts/Total Counts) X 100

   This should be 35-50%.

4. Calculate the percentage of total binding (%B/Bo) for each standard and sample:
   %B/Bo = (Sample or Standard/Total Binding) X 100

5. Plot the % B/Bo for each standard on the y-axis and the known concentration of the standard on the x-axis using log-log graph paper.

6. Construct the reference curve by joining the points with a smooth curve.

7. Determine the µU/mL of Human Insulin in the unknown samples and controls by interpolation of the reference curve.

   NOTE: When sample volumes assayed differ from 100 µL, an appropriate mathematical adjustment must be made to accommodate for the dilution factor (e.g., if 50 µL of sample is used, then calculated data must be multiplied by 2).

   Conversion to SI units
   1 µU Insulin / mL = 6 pM

X. INTERPRETATION

Acceptance Criteria

1. The run will be considered accepted when all Quality Control values fall within the calculated Quality Control Range; if any QC's fall outside the control range, review results with supervisor.

2. If the difference between duplicate results of a sample is >10% CV, repeat the sample.

3. The limit of sensitivity for the Human Insulin assay is 2.715 µU/mL (100 µL sample size).

4. The limit of linearity for the Human Insulin assay is 200 µU/mL (100 µL sample size). Any result greater than 200 µU/mL should be repeated on dilution using Assay Buffer as a diluent.

XI. NORMAL FASTING RANGE

5-15 µU/mL

This range was determined from the analysis of blood drawn from 25 people after an 18 hour fast.

XII. ASSAY CHARACTERISTICS

A. Sensitivity

The lowest level of insulin that can be detected by this assay is 2.715 µU/mL when using a 100 µl sample size.

B. Performance

The following parameters of assay performance are expressed as Mean ± Standard Deviation.

ED_{50} = 7 ± 1 µU/mL
ED_{80} = 26 ± 3 µU/mL
ED_{20} = 102 ± 10 µU/mL

C. Specificity

The specificity (also known as selectivity) of an analytical test is its ability to selectively measure the analyte in the presence of other like components in the sample matrix.

Human Insulin	100%
Human Proinsulin (HPI)	<0.2%
Des 31,32 HPI	<0.2%
Des 64,65 HPI	76%
Canine Insulin	100%
Porcine Insulin	100%
Bovine Insulin	62%
Rat Insulin	0.1%
IGF	ND
Glucagon	ND
Somatostatin	ND
Pancreatic Polypeptide	ND

ND-not detectable

XII. ASSAY CHARACTERISTICS (continued)

D. Precision

Within and Between Assay Variation

Sample No.	Mean µU/mL	Within % CV	Between % CV
1	8	3.1	6.0
2	12	2.5	3.3
3	16	2.2	3.8
4	25	3.8	2.9
5	54	4.4	3.4

Within and between assays variations were performed on five human serum samples containing varying concentrations of Human Insulin. Data (mean and % CV) shown are from five duplicate determinations of each serum sample in five separate assays.

E. Recovery

Spike & Recovery of Insulin in Human Serum

Sample No.	Insulin Added µU/mL	Observed µU/mL	Expected µU/mL	% Recovery
1	0	8	-	-
2	5	13	13	100
3	10	17	18	94
4	20	26	28	93
5	50	56	58	97

Varying concentrations of Human Insulin were added to five human serum samples and RIA determined the insulin content. Mean of the observed levels from five duplicate determinations in five separate assays are shown. Percent recovery was calculated on the observed vs. expected.

XII. ASSAY CHARACTERISTICS (continued)

F. Linearity

Effect of Serum Dilution

Sample #	Volume Sampled	Observed µU/mL	Expected µU/mL	% Of Expected
1	100 µl	17	17	100
	75 µl	15		88
	50 µl	15		88
	25 µl	15		88
2	100 µl	42	42	100
	75 µl	40		95
	50 µl	39		93
	25 µl	36		86
3	100 µl	62	62	100
	75 µl	59		95
	50 µl	57		92
	25 µl	55		89
4	100 µl	83	83	100
	75 µl	86		104
	50 µl	87		105
	25 µl	90		108

Aliquots of pooled Human Serum containing varying concentrations of Insulin were analyzed in the volumes indicated. Dilution factors of 1, 1.33, 2 and 4 representing 100 µL, 75 µL, 50 µL and 25 µL, respectively, were applied in calculating observed concentrations. Mean insulin levels and percent of expected for five separate assays are shown.

XII. ASSAY CHARACTERISTICS (continued)

G. Example of Assay Results

This data is presented as an example only and should not be used in lieu of a standard curve prepared with each assay.

Tube #	ID	CPM	Ave CPM	Ave Net CPM	% B/Bo	µU/mL
1	Totals	14879				
2	"	15738	15309			
3	NSB	683				
4	"	604	644			
5	Bo	5836				
6	"	5808	5822	5179		
Standards						
7	3.125 µU/mL	5262				
8		5039	5151	4507	87.0	
9	6.25 µU/mL	4530				
10		4666	4598	3955	76.4	
11	12.5 µU/mL	3949				
12		3775	3862	3219	62.2	
13	25 µU/mL	3100				
14		3067	3084	2450	47.3	
15	50 µU/mL	2006				
16		2100	2053	1410	27.2	
17	100 µU/mL	1426				
18		1477	1452	808	15.6	
19	200 µU/mL	1053				
20		997	1025	382	7.4	
Controls/Unknown						
21	QC 1	4033				
22		3999	4016	3373	65.1	11.4
23	QC 2	2075				
24		2157	2116	1473	28.4	48.86
25-n	Unknown					

XIII. QUALITY CONTROLS

Good Laboratory Practice (GLP) requires that Quality Control (QC) specimens be run with each standard curve to check the assay performance. Two levels of controls are provided for this purpose. Quality control data is provided on an insert sheet within the protocol booklet. These quality controls and any other control materials should be assayed repeatedly to establish mean values and acceptable ranges. Each individual laboratory is responsible for defining their system for quality control decisions and is also responsible for making this system a written part of their laboratory manual. The ranges for Quality Control 1 and 2 are provided on the card insert or can be located at the Millipore website www.millipore.com/bmia.

Recommended batch analysis decision using two controls (Westgard Rules):[4]

1. When both controls are within ±2 SD.
   Decision: Approve batch and release analyte results.

2. When one control is outside ±2 SD and the second control is within ±2 SD.
   Decision: Hold results, check with supervisor. If no obvious source of error is identified by the below mentioned check of systems, the supervisor may decide to release the results.

   Technician check of systems:
   1. Check for calculation errors
   2. Repeat standards and controls
   3. Check reagent solutions
   4. Check instrument

XIV. REPLACEMENT REAGENTS

Reagent	Cat#
^{125}I-Insulin (<5 µCi, 185 kBq)	9011
Label Hydrating Buffer (27 mL)	LHB-P
Human Insulin Standards (2 mL each)	8014-K
Human Insulin Antibody (26 mL)	1014-K
Precipitating Reagent (260 mL)	PR-UV
QC 1&2 (1 mL each)	6000-K
Assay Buffer (40 mL)	AB-P

XV. ORDERING INFORMATION

A. To place an order:

For USA Customers:

Please provide the following information to our customer service department to expedite your telephone, fax or mail order:

1. Your name, telephone and/or fax number
2. Customer account number
3. Shipping and billing address
4. Purchase order number
5. Catalog number and description of product
6. Quantity and product size
   NOTE: Appropriate license from NRC (or equivalent) must be on file at Millipore before radioactive orders can be shipped.

TELEPHONE ORDERS:
Toll Free US (800) MILLIPORE
FAX ORDERS: (636) 441-8050
MAIL ORDERS: Millipore
6 Research Park Drive
St. Charles, Missouri 63304 U.S.A.

For International Customers:
To best serve our international customers, it is Millipore's policy to sell our products through a network of distributors. To place an order or to obtain additional information about Millipore products, please contact your local distributor.

B. Conditions of Sale

All products are for research or manufacturing use only. They are not intended for use in clinical diagnosis or for administration to humans or animals. All products are intended for *in vitro* use only.

C. Material Safety Data Sheets (MSDS)

Material safety data sheets for Millipore products may be ordered by fax or phone. See Section A above for details on ordering.

XVI. REFERENCES

1. Morgan, C.R. and Lazarow, A. Immunoassay of Insulin: Two antibody system. Plasma insulin levels in normal, Subdiabetic, and diabetic rats. Diabetes 12:115-126, 1963.

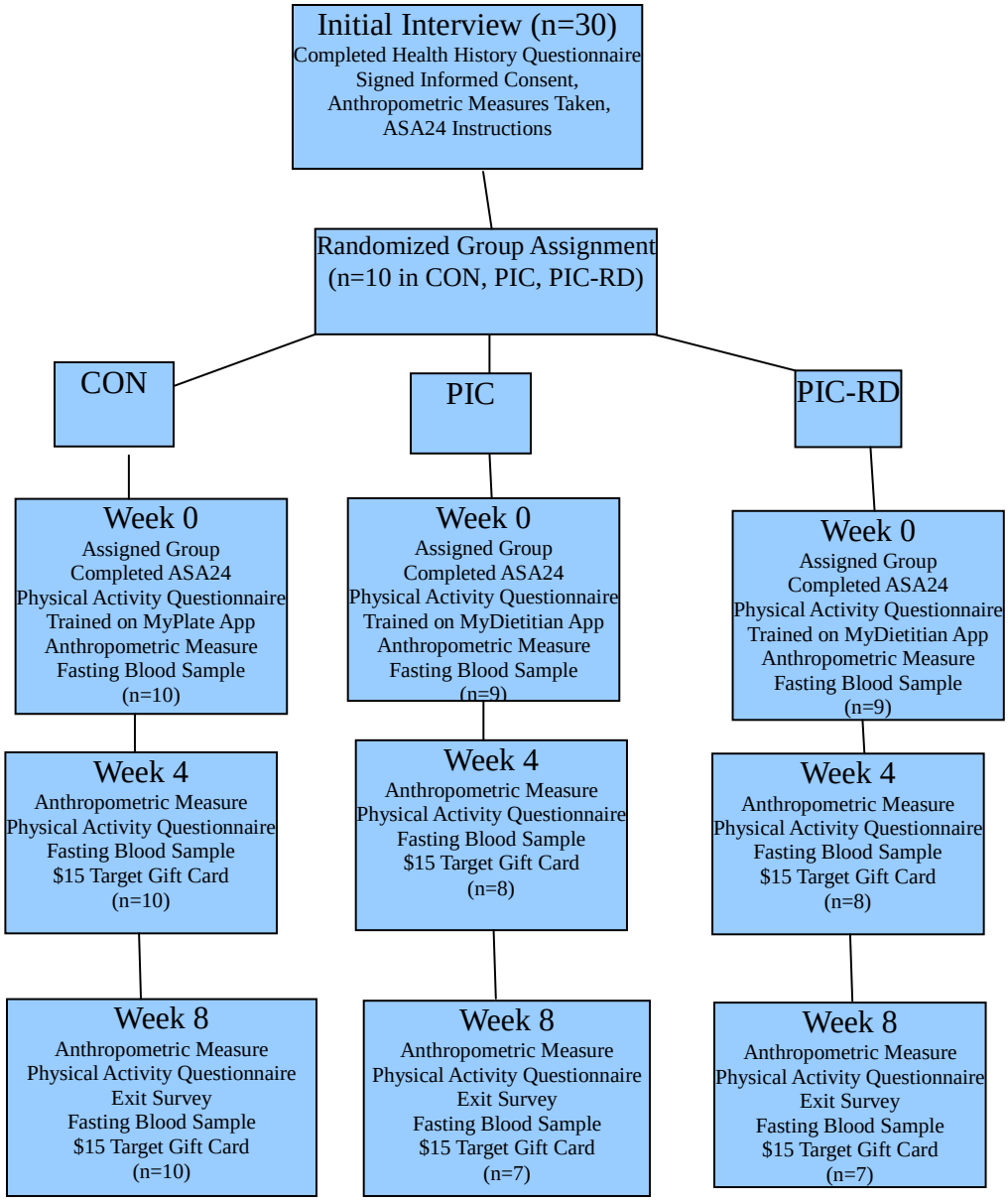
2. Thorell, J.I. *Scand. J. Clin. Lab. Invest.* 31:187, 1973.

3. Feldman, H. and Rodbard, D. "Mathematical Theory of Radioimmunoassay," in: W.D. Odell and Doughaday, W.H. (Ed.), Principles of Competitive Protein-Binding Assays. Philadelphia: J.B. Leppincott Company; pp 158-203, 1971.

4. Westgard, J.O., et. al. A multi-rule Shewhart chart for quality control in clinical chemistry. *Clin. Chem.* 27:493-501, 1981.

APPENDIX R

TIMELINE


Initial Interview (n=30)
Completed Health History Questionnaire
Signed Informed Consent,
Anthropometric Measures Taken,
ASA24 Instructions
Randomized Group Assignment
(n=10 in CON, PIC, PIC-RD)
CON
PIC
PIC-RD
Week 0
Assigned Group
Completed ASA24
Physical Activity Questionnaire
Trained on MyPlate App
Anthropometric Measure
Fasting Blood Sample
(n=10)
Week 0
Assigned Group
Completed ASA24
Physical Activity Questionnaire
Trained on MyDietitian App
Anthropometric Measure
Fasting Blood Sample
(n=9)
Week 0
Assigned Group
Completed ASA24
Physical Activity Questionnaire
Trained on MyDietitian App
Anthropometric Measure
Fasting Blood Sample
(n=9)
Week 4
Anthropometric Measure
Physical Activity Questionnaire
Fasting Blood Sample
$15 Target Gift Card
(n=10)
Week 4
Anthropometric Measure
Physical Activity Questionnaire
Fasting Blood Sample
$15 Target Gift Card
(n=8)
Week 4
Anthropometric Measure
Physical Activity Questionnaire
Fasting Blood Sample
$15 Target Gift Card
(n=8)
Week 8
Anthropometric Measure
Physical Activity Questionnaire
Exit Survey
Fasting Blood Sample
$15 Target Gift Card
(n=10)
Week 8
Anthropometric Measure
Physical Activity Questionnaire
Exit Survey
Fasting Blood Sample
$15 Target Gift Card
(n=7)
Week 8
Anthropometric Measure
Physical Activity Questionnaire
Exit Survey
Fasting Blood Sample
$15 Target Gift Card
(n=7)